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ISBN 0-315-56353-2

Présence de canaux activés par l'étirement dans les neurones de l'escargot terrestre, *Cepaeae nemoralis*.

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Thèse déposée à
l'Ecole des études supérieures et de la recherche en vue de l'obtention de la maîtrise ès science en biologie

Ottawa, Ontario, 1988

Université d'Ottawa/University of Ottawa

UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

A Loretta

REMERCIEMENTS

J'aimerais remercier le Dr. Cathy Morris pour m'avoir prêté conseil tout au long de ma recherche et lors de la revision de cette thèse. Je tiens aussi à remercier M. Wade Sigurdson pour son aide technique. Un merci spécial à Loretta Roy et Anne Bouillon pour leur support moral.

RESUME

Un canal de K sensible à l'étirement (canal SAK) a été trouvé dans les corps cellulaires des cellules nerveuses de l'escargot terrestre Cepaea nemoralis (famille Hélicidée). Ce canal dont la demi-saturation de pression est de -55 mm de Hg, possède une conductance de 58 pS dans des conditions cellule-attachée avec une solution saline normale dans la pipette. Ce canal dont la perméabilité au Na est non-détectable, est bloqué du côté cytoplasmique de la membrane par ce dernier (80 mM Na). Le canal SAK est insensible à 10^{-3} M TEA (tétraéthylamonium) externe, 10^{-3} M 4-AP (4-aminopyridine) externe, au Ca et au Na. Il est cependant affecté par 10^{-3} M quinidine. Ce canal possède une constante de temps pour l'état ouvert et trois constantes de temps pour l'état fermé. Le plus long état fermé est responsable de l'augmentation de la probabilité du canal d'etre à l'état ouvert avec l'application de suction.. Un canal d'étirement sélectif au Cl (canal SACl) a aussi été observé dans les neurones de Cepaea. Contrairement au canal SAK, le canal SACl est non-flickery et possède plusieurs états de conductance. La conductance de l'état le plus souvent observé est de 130 pS. Des pressions de

l'ordre de -35 à -40 mm de Hg permettent l'ouverture du canal. Aucun canal d'étirement n'a été observé dans les corps cellulaires des neurones de la sangsue et de la blatte.

ABSTRACT

A stretch-activated channel selective for K (SAK channel) has been discovered in the cell boby of the nerve cell of the terrestrial snail Cepaea nemoralis (Helicidae family). The channel has a half pressure saturation of -55 mm Hg. Its conductance is 58 pS in cell-attached condition with normal saline in the pipette. The SAK channel which permeability to Na is undetectable, is blocked by Na ion (80 mM Na) from the cytoplasmic side of the membrane. 10^{-3}M external TEA (tetraethylammonium), 10^{-3} M 4-AP (4-aminopyridine), Ca and Na do not affect the channel activity. However, 10^{-3} M quinidine causes changes in SAK channel activity. The channel has one time constant for the open state and three time constants for the closed state. The longer closed state is responsable for the large increase in the channel probability of being opened with suction application. A stretch channel selective for Cl (SACl) has also been found in Cepaea neurons. Contrary to the SAK channel, the SACl is non-flickery and has many conductance states. The conductance of the most observed state is 130 pS. Pressures in the range of -35 to -40 mm Hg trigger the SACl channel

opening. No stretch-activated channel has been observed in the cell body of the leech and cockroach neurons.

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CHAPITRE I

I INTRODUCTION

Les canaux ioniques sont des pores protéiques transmembranaires qui permettent la diffusion d'ions suivant leur gradient électrochimique (Hille, 1984). La sélectivité spécifique des canaux c'est-à-dire le fait qu'ils soient perméable à certains ions non à tous les ions confère à ces derniers un rôle spécifique à jouer dans la physiologie cellulaire. Le maintien du potentiel de repos ou encore la génération et la propagation de l'impulsion électrique des cellules nerveuses ne seraient possibles sans la sélectivité spécifique des canaux membranaires au K et au Na (Kandel and Schwartz, 1983).

En plus d'être sélectifs, les canaux ioniques ont la propriété d'être modulables c'est-à-dire qu'ils répondent (par une ouverture ou une fermeture) de façon spécifique à certains stimuli. La modulation du canal peut être électrique (ex.: canaux de K dépendant du voltage; Quandt, 1988), chimique (ex.: canaux d'acétylcholine; Fenwick, Marty and Neher, 1982; Mathers and Barker, 1982) ou mécanique (canaux activés par

l'étirement; Guharay and Sachs, 1984). C'est de cette dernière catégorie de canaux dont il sera question dans ce qui suit.

I.1 Historique

Contrairement aux autres techniques d'enregistrement de courants (voltage-clamp; Hodgkins and Huxley, 1939), la technique du patch-clamp (Hamill, Marty, Neher, Sakmann and Sigworth, 1981; Sakmann and Neher, 1983), en plus de permettre l'enregistrement de courants distincts passant à travers les canaux ioniques, permet l'application d'une succion sur la membrane cellulaire. Grâce à cette combinaison technique, l'identification directe du premier canal activé mécaniquement a été possible.

Alors qu'ils étudiaient des canaux nicotiniques dans les cellules des muscles squelettiques d'embryon de poulet, Guharay et Sachs observèrent que l'activité de l'un des canaux présent dans les patches augmentait avec l'application de succion (Guharay and Sachs, 1984). La relation probabilité du canal d'être à

l'état ouvert, P(O), versus pression révéla qu'une augmentation de la pression appliquée entraînait une augmentation nette de P(O). Des expériences contrôles faites en absence de nicotine, avec de basses et de hautes concentrations de calcium (20 mM - 10^{-9} M), en absence de machinerie enzymatique ou de d'autres métabolites ("inside-out patches") démontrèrent que la sensibilité du canal à l'étirement était toujours présente et qu'elle n'était pas affectée par les facteurs ci-haut (Guharay and Sachs, 1984). Une caractérisation plus approfondie du canal d'étirement révéla que ce dernier était un canal cationique perméable au K et au Na (K:Na = 4:1) (Guharay and Sachs, 1984).

Depuis l'identification du canal d'étirement (CE) dans les cellules musculaires de poulet de nombreux canaux activés par l'étirement ont été découverts et ce, dans divers types de cellules de différentes espèces animales (Kullberg, 1987; Lansman, 1988). La découverte de plus en plus fréquente de canaux d'étirement nous amène à considérer ces derniers comme la troisième classe de canaux membranaires.

I.2 Mécanisme d'ouverture des canaux d'étirement

L'activité spontanée des CE est basse lorsqu'il n'y a pas application de succion (Guharay and Sachs, 1984; Brezden, Gardner and Morris, 1986; Methfessel, Witzemann, Takahashi, Mishina, Numa and Sakmann, 1986; Christensen, 1987; Lansman, Hallam and Rink, 1987; Martinac, Buechner, Delcour, Adler and Kung, 1987; Zagotta, Brainard and Aldrich, 1988). Cette faible activité est due à l'agitation continuelle du canal par des facteurs thermiques. Afin d'augmenter l'activité du canal, un transfert d'énergie mécanique doit s'effectuer entre la membrane et le canal. Le mécanisme le plus propice à un tel transfert serait le cytosquelette (Sachs, 1987). Parmi les éléments du cytosquelette, l'actine semblait être l'élément le plus probable au transfert d'énergie. Cependant, des expériences faites sur des cellules musculaires de poulet traitées à la cytochalasine (10^{-5} M pour 12 heures; drogue brisant les filaments d'actine) ont démontré que contrairement à ce qui était attendu, la sensibilité à l'étirement du CE était augmentée d'un facteur de 30 (Guharay and Sachs, 1984). Suite à ces

observations, Guharay et Sachs ont suggéré que des filaments lâches du cytosquelette tels la fodrine (retrouvée dans les neurones; Siman, Baudry and Lynch, 1985), filament semblable à la spectrine (présent dans les globules rouges; Fowler. 1986), pourraient être responsables de l'ouverture des CE. Des études à venir diront si un tel filament est impliqué ou non dans le mécanisme d'ouverture des CE.

I.3 Fonctions des canaux d'étirement

I.31 Mécanotransduction

L'élément responsable de la transduction de l'énergie mécanique en énergie électrique se produisant dans plusieurs organes de réception pourrait être le canal d'étirement. Dans les cellules ciliées du système auditif, le déplacement des cils, sous l'effet des vibrations de la membrane basilaire (membrane supportant les cellules ciliées et dont les vibrations sont causées par le déplacement du fluide de l'oreille interne sous l'influence des ondes sonores; Hudspeth, 1983) pourrait permettre l'ouverture d'un canal

d'étirement et ainsi causer l'entrée de K nécessaire au processus de génération de l'impulsion nerveuse dans le nerf auditif. Dans les fuseaux musculaires de grenouille (Husmark and Ottoson, 1971) et dans les organes récepteurs de l'écrevisse (Pasztor and Bush, 1987), le CE pourrait permettre la génération du potentiel récepteur observé lors de l'étirement de la membrane de ces deux organes. De même, la génération d'un tel potentiel dans les récepteurs nerveux des corpuscules de Pacinie (Lowenstein and Mendelson, 1965) pourrait être expliquée par l'activation d'un CE suite à l'application d'une pression sur le corpuscule.

Les CE pourraient être les canaux des barorécepteurs permettant la détection de la pression interne du système circulatoire et du système respiratoire (Richter, Jordan, Ballantyne, Meesmann and Spyer, 1986). Dans les cellules endothéliales recouvrant la surface interne des vaisseaux sanguins, un canal d'étirement sélectif aux cations (Lansman et al., 1987), de même qu'un courant de K activé par une force tangentielle (Olesen, Chapman and Davies, 1988) ont été identifiés. Sous l'influence du flux sanguin, l'activation du CE cationique pourrait permettre

l'entrée de Ca nécessaire au relâchement des facteurs requis pour la contraction des muscles lisses des vaisseaux sanguins (Lansman et al., 1987). D'une façon similaire, la sortie de K, probablement causée par l'activation d'un CE , pourrait permettre la dilatation des vaisseaux sanguins (Olesen et al., 1988). Ainsi, en détectant la pression interne, les CE pourraient changer la pression sanguine et contrôler le flux sanguin local des vaisseaux sanguins.

I.32 Régulation du volume

Les CE pourraient être impliqués dans la régulation du volume cellulaire (Guharay and Sachs 1984; Brezden et al., 1986; Cooper, Tang, Rae and Eisenberg, 1986; Martinac et al., 1987; Sigurdson, Morris, Brezden and Gardner, 1987) et ainsi prévenir l'éclatement de la cellule. Sous l'influence d'un stress hypoosmotique, l'étirement de la membrane résultant du gonflement de la cellule pourrait activer les CE et permettre la sortie d'ions accompagnée de perte d'eau. Dans les cellules rénales d'opussum, une augmentation de l'activité d'un CE sélectif au Cl^- a été observée pendant le gonflement de la cellule sous choc

hypoosmotique (Ubl, Murer and Kolb, 1988). Le rétablissement des conditions osmotiques originelles (milieu normal dans lequel la cellule vit) a entrainé une diminution de l'activité du CE et ramené son activité à son niveau initial. Dans les cellules épithéliales du plexus choroïdien de la salamandre Necturus maculosa, l'activation de CE cationiques (permettent l'entrée de Ca) entraîne l'activation de canaux de K dépendant du Ca et du voltage, ce qui pourrait permettre une diminution du volume cellulaire (Christensen, 1987).

La régulation du volume cellulaire était probablement l'un des principaux mécanismes de survie chez les premiers organismes à apparaître. Il est donc possible que les canaux CE impliqués dans l'osmorégulation aient été l'une des premières classes de canaux à évoluer (Kullberg, 1987). Des CE ont d'ailleurs été retrouvés chez des microorganismes tels la levure Saccharomyces cerevisiae (Martinac, Gustin, Zhou, Culbertson, Buechner, Delcour, Adler and Kung, 1988) et Escherichia coli (Martinac et al., 1987).

I.33 Réponse aux obstacles

Les CE pourraient avoir un rôle à jouer dans le comportement de microorganismes tels la Paramécie. Lorsque cette dernière est en contact avec un obstacle, la pression exercée sur la cellule permet une entrée de calcium, ce qui change la direction de battement des cils et permet à l'animal d'aller dans la direction opposée (Otter and Salmon, 1985). Les CE pourraient être impliqués dans la réponse de la cellule à la pression.

I.4 Propriétés générales des CE

Les CE sont activés par des pressions tant négatives que positives. Des pressions situées entre -10 et -70 mm de Hg permettent l'activation des CE (la littérature ne donne pas de valeurs de pression positive; Guharay and Sachs, 1984; Brezden et al., 1986; Cooper et al., 1986; Methfessel et al., 1986; Christensen, 1987; Lansman et al., 1987; Martinac et al., 1987; Sigurdson et al., 1987). Ils sont insensibles au Ca et sont légèrement affectés par une dépolarisation (Methfessel

et al., 1987; Martinac et al., 1987). Leurs conductances se situent entre 30 et 100 pS (pour 100 à 105 mM de Na ou K ou Cl) (Guharay and Sachs, 1984; Brezden et al., 1986; Cooper et al., 1986; Methfessel et al., 1986; Christensen, 1987; Lansman et al., 1987; Sigurdson et al., 1987; Zagotta et al., 1988). Ils possèdent un ou deux états de conformation pour l'état ouvert et de trois à quatre conformations pour l'état fermé dont le plus long de ces derniers est affecté par l'étirement (Guharay and Sachs, 1984; Christensen, 1987; Lansman et al., 1987; Sigurdson et al., 1987). Ces canaux sont uniformément distribués et leur densité varie entre 0.5 et 2 canaux/μm^2 (Brezden et al., 1986; Methfessel et al., 1986; Christensen, 1987).

I.5 Sélectivité des CE

Trois types de canaux activés par l'étirement ont été identifiés suivant leur sélectivité: le CE sélectif aux cations, le CE sélectif aux anions et le CE sélectif au K.

I.51 CE sélectif aux cations (stretch-activated cation channel; SA)

Le CE sélectif aux cations est perméable au Na, K et Ca (Guharay and Sachs, 1984; Cooper et al., 1986; Methfessel et al., 1987; Christensen, 1987; Lansman et al., 1987). La perméabilité au K est toujours supérieure à celle du Na et du Ca alors que l'ordre de perméabilité de ces deux derniers varie d'un type de cellule à l'autre. Ce canal est retrouvé dans différents tissus de vertébrés tels les cellules musculaires de poulet (Guharay and Sachs, 1984), l'épithélium de la lentille de grenouille Xenopus laevis (Cooper et al., 1984), le plexus choroïdien de la salamandre Necturus maculosa (Christensen, 1987), les cellules endothéliales vasculaires de porc (Lansman et al., 1987) et les oocytes de Xenopus laevis (Methfessel et al., 1987).

I.52 CE sélectifs aux anions (stretch-activated anion channel)

Le CE sélectif aux anions est principalement sélectif au Cl (Martinac et al., 1987; Martinac et al, 1988;

Ubl et al.; 1988). Contrairement à ce que leur nom suggère, ils sont parfois perméables à certains cations tels Na, K et Ca. Cependant, la perméabilité à ces cations est plus petite que celle aux anions (Martinac et al., 1987; Mactinac et al., 1988). L'un de ces CE sélectif aux anions possède plusieurs états de sous-conductance et a une conductance de 900 pS (Martinac et al., 1987). Le CE anionique est présent chez les vertébrés (cellules rénales d'opossum; Ubl et al, 1988) et chez les microorganismes (Saccharomyces cerevisiae; Martinac et al., 1988; Escherichia coli; Martinac, et al, 1987).

I.53 CE sélectif au K (stretch-activated K channel; SAK)

Le dernier type de CE et non le moindre, soit le CE sélectif au K, sera le point d'intérêt de cette présente thèse. Au début de cette recherche, ce canal hautement sélectif au K et légèrement perméable au Na (K:Na=16:1; Brezden al., 1986) était particulier due au fait qu'il n'avait été identifié que chez une seule espèce animale, soit l'escargot d'eau douce Lymnaea

stagnalis. Aucun CE sélectif au K n'avait été observé chez les vertébrés et microorganismes. Cependant, cette situation a quelque peu changé comme nous le verrons plus loin. Chez cet animal, la fonction du canal SAK semble générale étant donné la présence de ce dernier dans différents types de cellules tels les cellules cardiaques, nerveuses et rénales (Brezden et al., 1986; Sigurdson et al., 1987). Il a été mentionné que le canal SAK pourrait être impliqué dans la régulation du volume cellulaire (Brezden et al., 1986; Sigurdson et al,. 1987).

I.6 Objectifs

L'objectif de cette thèse est de cherher des CE sélectifs au K dans différentes espèces animales.

Les animaux choisis devaient répondre aux trois critères suivants:

i) l'animal devait être un invertébré étant donné que le canal avait été identifié seulement chez un invertébré.

ii) l'animal devait appartenir au même phylum que Lymnaea stagnalis ou à un phylum voisin. Les chances de trouver le canal SAK s'en trouvaient augmentées.

iii) l'un des animaux choisis devait vivre dans le même milieu que Lymnaea soit le milieu aquatique et les deux autres devaient vivre dans des environnements différents, soit terrestre ou marin. Le type d'environnement dans lequel serait trouvé le canal SAK pourrait donner des indications concernant sa fonction.

I.7 Espèces animales choisies

Etant donné le temps alloué à ce projet, trois espèces animales ont été choisies.

I.71 Phylum: Mollusque

Représentant: Cepaea nemoralis (famille des Hélicidés)

Milieu de vie: terrestre

Particularité: facile à obtenir et à cultiver; appartient à la même famille qu'Hélix, un hélicidé communément utilisé en neurophysiologie (Gerschenfeld, Hammond and Pampardin-Tritsch, 1986;Partridge and Swandulla, 1987).

I.72 Phylum: Annélides (situé au-dessus des Gastropodes dans l'échelle évolutive)

Représentant: Sangsue, Hirudo medicinalis

Milieu de vie: aquatique (peut survivre en milieu terrestre)

Particularité: l'étirement des parois corporelles permet la génération d'un courant dans les neurones afférentes innervant ces dernières (Blackshaw, 1985); animal communément utilisé en

neurophysiologie (Ready and Nicholls, 1979; Gerasimov, 1968).

I.73 Phylum: Gastropode (phylum situé au-dessus des Annélides)

Représentant: Blatte, Periplanata americana

Milieu de vie: terrestre

Particularité: facile à obtenir et à cultiver; animal communément utilisé en neurophysiologie (Beadle, Pichon and Takeshi, 1986; French, 1986).

N.B. Le type de cellule choisi pour la recherche du canal SAK est le neurone. Trois raisons ont suscité ce choix:

i) la grosseur des cellules.

ii) leur facilité d'isolation. Le traitement permettant la séparation des cellules et le nettoyage de leur surface membranaire est généralement de courte durée et peu compliqué.

iii) leur facilité à être patchée. Le nettoyage de la surface membranaire des neurones rend ces dernières généralement faciles à patcher.

I.8 Atteintes des objectifs

Les objectifs de cette thèse seront atteints en analysant les canaux ioniques présents dans les neurones des différentes espèces animales mentionnées ci-haut. L'analyse sera faite comme suit:

I.81 Détermination de la sensibilité à l'étirement du canal.

Pour les canaux identifiés comme étant sensibles à l'étirement,

I.8ii la sélectivité ionique sera déterminée

I.8iii divers modulateurs seront testés

I.8iiii le nombre d'états de conformation ouverts fermés ainsi que l'état affecté par l'étirement seront déterminés.

I.82 Absence de canaux d'étirement.

Si aucun CE n'est observé, une courte caractérisation des autres canaux présents dans les cellules sera effectuée afin de démontrer que l'absence de CE n'est pas due à des conditions d'enregistrement inappropriées.

CHAPITRE II

II MATERIALS AND METHODS

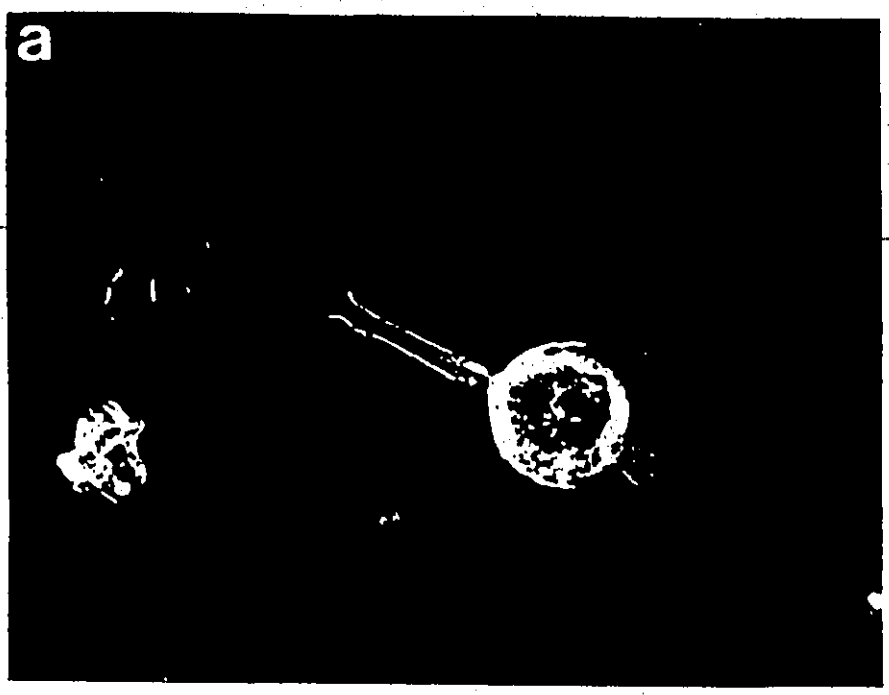
II.1 Cell isolation

In order to do single channel recording, a high resistance seal (10^9 Ohms called gigaseal) between the pipette and the membrane must be established. To facilitate the formation of gigaseals, cells are isolated and allowed to attach to a transparent surface (eg. dish in which they are cultured); most important, however, the cell membrane must be cleaned. Cell membrane cleaning and cell isolation can be done by enzymatic treatment and mechanical agitation whereas cell attachment can be facilitated by using various adhesion surfaces (glass, plastic) coated or not with chemicals such as, for example, poly-L-lysine or collagen. The following cell treatments were done to obtain appropriate cells for single channel recordings.

II.11 *Cepaea nemoralis*

The suboesophagal ganglia were dissected under aseptic conditions. The enzymatic treatment was found to be ineffective for the cerebral ganglion, so these were not used. To achieve uniform digestion, the connective tissue sheath surrounding the ganglia was disrupted and the ring of ganglia was cut to form a chain. The chain was then cut in two pieces and put in a tube containing 1.5 ml of 0.25% protease (Sigma, type XIV). After an hour agitation, the ganglia were pipetted and put in a petri dish containing sterile NCS (normal *Cepaea* saline; Trams, Lauter, Bourkes and Towers, 1965; Table 1) to which had been added 1% of a solution of streptomycin/ penicillin (Sigma). The remaining connective tissue was removed with scissors and forceps, leaving the ganglia naked; cells were then dispersed with two fine needles. Small and large nerve cells (diameter, 30-80 µm) with or without neurites were used for patch recording one, two or three days after isolation. About 10% of the cells formed neurites after one day (fig.1). Currents due to action potentials could occasionaly be seen when patches were performed.

Fig.1 **Isolated *Cepaea nemoralis* neurons.** a) 2 day neuron with neurites and filopodia. b) 3 day neuron which has not formed neurites. Magnification, 400X.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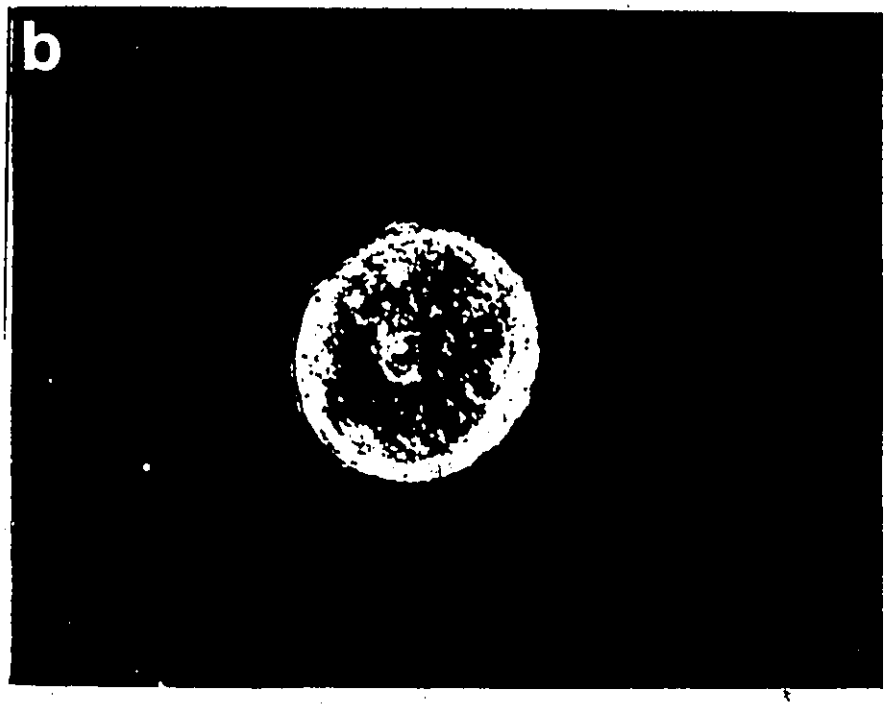
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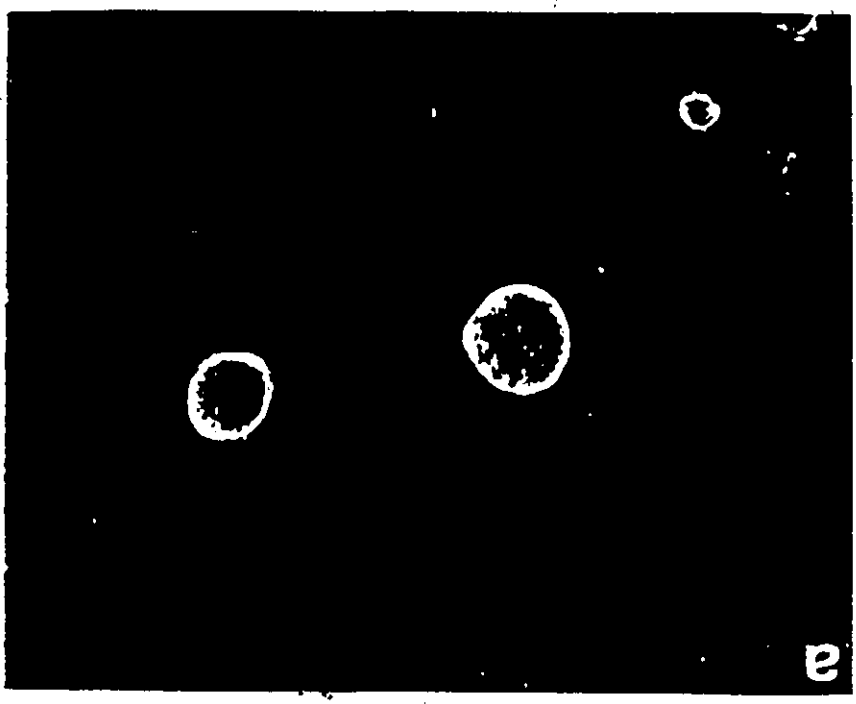
II.12 Leech (*Hirudo medicinalis*)

A chain of several ganglia of the ventral nerve cord was removed and dissected away from the sinus venosus surrounding it under aseptic conditions. With fine needles, the connective tissue capsule was slightly broken allowing the nerve cells to pop out without detaching from the ganglia. The ganglia were transferred to a tube containing 2 ml of 0.25% protease (Sigma, type XIV). The cell were agitated 40 minutes and centrifuged 15 minutes at 115 rpm. The supernatant was replaced by 2 ml of Leibovitz L-15 medium with glutamine (Gibco Laboratories) and recentrifuged for 15 minutes. The pellet was then transferred to a glass coverslip coated with poly-L-polysine (Sigma). Cells still attached to the connective tissue capsule, were manually dispersed as in *Cepaea*. The cells were cultured in sterile Leibovitz L-15 medium at room temperature. Cells (diameter, 20-50 µm) were patched one day after isolation (the cell membrane after one day was not smooth and rendered gigaseal formation difficult). The smaller nerve cells were more firmly attached and were most easily patched (fig.2). Gigaseals were difficult to obtain with giant neurones due to the poor attachment of large neurones to the

Fig.2 Isolated leech and cockroach neurons. a) 1 day leech neuron. b) 1 day cockroach neuron which has started to form neurites. Magnification 400X.


q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dish and to the presence of debris on the membrane (probably from damaged glial cells). Few cells formed neurites.

II.13 Cockroach (*Periplanata americana*)

The abdominal nerve cord of the last instar cockroach was removed and the connective tissue surrounding the ganglia was disrupted to facilitate the enzymatic action on the neuronal surfaces. The ganglia were agitated for 40 minutes in 2 ml of 0.25% protease (Sigma, type XIV) after which the connective tissue was removed. The cells were then dispersed with needles in a petri dish containing sterile NPS (normal *Periplanata* saline; Beadle, Pichon and Takeshi, 1986; Table 2). They were cultured in NPS to which had been added 1% of a solution of streptomycin/penicillin (Sigma). Large and small cells (diameter, 20-50 µm) were patched the day following isolation (fig.2). Few cells formed neurites.

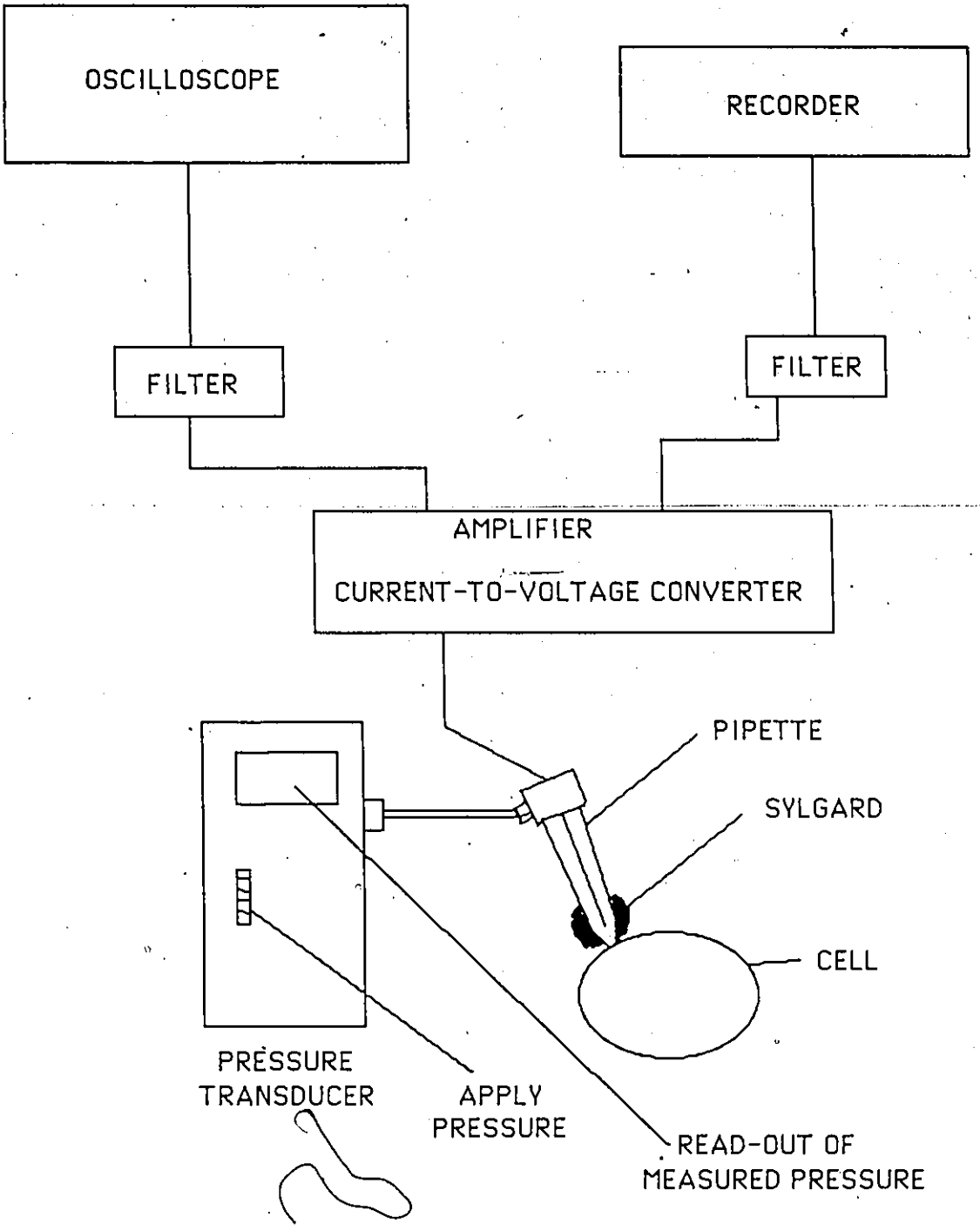
II.2 Patch-clamp technique

A general view of the patch-clamp apparatus is presented in figure 3. Once a gigaseal between the cell membrane and the pipette has been formed, single channel current in the order of picoamperes (10^{-12} A) is amplified prior to being recorded. Currents are usually filtered prior to recording. While making single channel recordings, an oscilloscope is used to monitor the progress of the experiment.

Single channel currents were recorded using standard patch clamp techniques (Hamill et al., 1981), using either an Axopatch instrument or List EPC-7 patch clamp amplifier. Currents were filtered using an 8 pole Bessel filter and recorded on an FM recorder (Racal Store 4) or VCR recorder (Sony Ba II) via a pulse code modulator, (PCMI Medical Systems, Greendale, N.Y.). Voice recordings were made routinely as part of the experimental record.

Patch electrodes were fabricated from borosilicate tubing (Corning 7052, Garner glass co., ID = 0.80 $\pm$ 0.05 mm, O.D. = 1.65 $\pm$ 0.05 mm) with a programable

Fig.3 View of the patch-clamp apparatus. Single channel currents are amplified before being viewed on an oscilloscope and recorded for further analysis. Pressure is applied to the membrane with a pressure transducer connected to the pipette holder. In order to decrease the inherent noise of the system, currents can be filtered prior to recording or viewing on the oscilloscope.


OSCILLOSCOPE
RECORDER
FILTER
FILTER
AMPLIFIER
CURRENT-TO-VOLTAGE CONVERTER
PIPETTE
SYLGARD
CELL
PRESSURE TRANSDUCER
APPLY PRESSURE
READ-OUT OF MEASURED PRESSURE

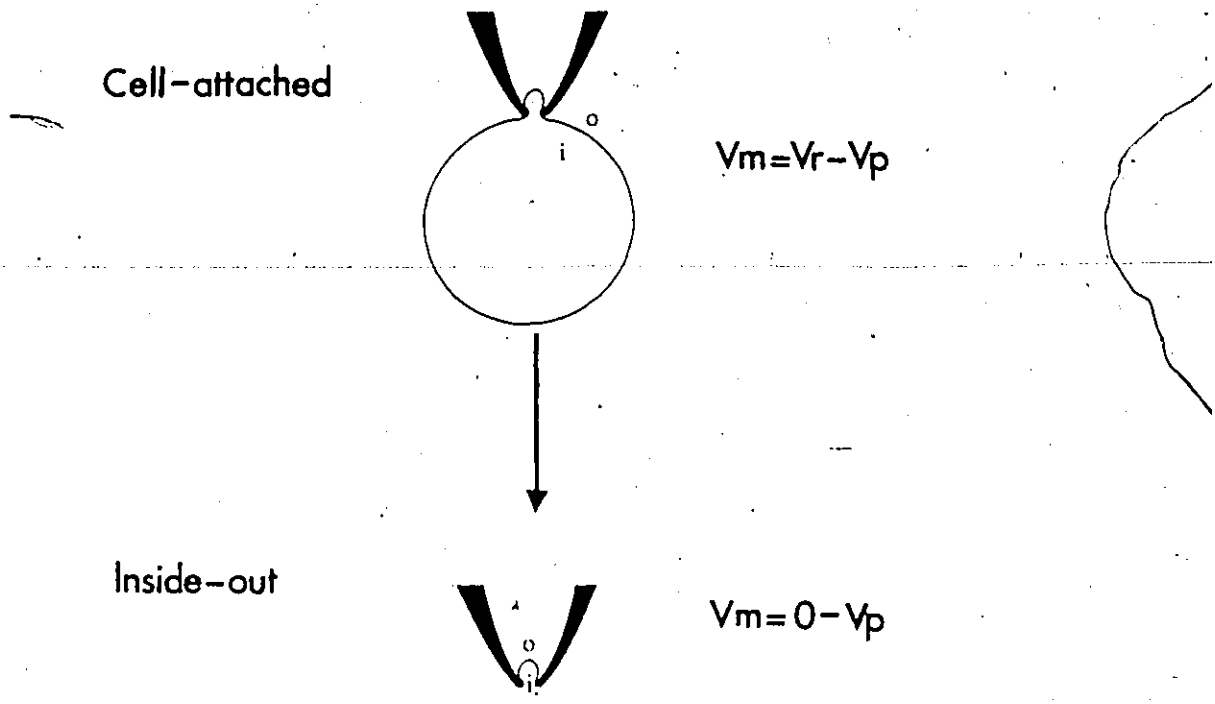
pipette puller (model 750, David KOPF instruments) and coated with Sylgard 184 (silicone elastomer; Dow Corning) to reduce pipette capacitance. The pipettes were firepolished to facilitate gigaseal formation. Pipette resistance ranged between 10 and 13 MOhms with 70 mM KCl in the pipette and NCS in the bath. Ag/AgCl electrodes were used in the micropipette and as a ground in the bath (fig. 3).

Negative or positive pressures were applied to the patch membrane with a pressure transducer (model DPM-1; Bio-tek) connected to the pipette holder via a plastic tube (fig.3). The same instrument measured the pressure in the system. When no pressure was applied, a port on the pressure transducer's three-way valve was opened to allow the patch to equilibrate to atmospheric pressure. The experiments were carried out at room temperature (20-24^{o}C).

II.3 Condition of recording

The experiments were carried out using two variants of patch-clamp method: cell-attached and the inside-out conditions (fig.4). In the first case the pipette is

FIG. 4 **Two variants of the patch-clamp technique:** cell-attached patch and inside-out patch.


Cell-attached
o
i
Vm=Vr-Vp
Inside-out
o
i
Vm=0-Vp

in contact with the neurone, and the potential across the patch membrane (Vm) is equal to the difference between the cell resting potential (Vr) and the pipette potential (Vp). The resting potentials for Cepaea, Hirudo and Periplanata neurons were -50 mV (Trams et al., 1965; Deitmer and Schlue, 1981; Leech, 1986) (In the inside-out experiments a patch of the membrane is isolated from the cell, and its internal side (cytoplasmic side) faces the bath solution. In this case, the membrane potential (Vm) is equal to difference between the ground (ie. 0 mV) and the pipette potential (Vp). Currents are considered positive when positive charge moves from the cytoplasm (cell-attached) or the bath (inside-out) to the external side of the membrane (pipette). Such positive currents are displayed as upward deflections, and vice versa for currents going from the pipette to the bath solution or into the cell.

II.4 Analysis of results

II.41 I/V curves

For current/voltage (I/V) relationships, generally 5 fully-resolved single channel currents were manually measured from records played back onto a storage oscilloscope. In cases where stretch-activated-ion channels were being studied, suction was applied to the membrane at each voltage to ensure that the measured event was in fact a stretch-sensitive one. Linear regression was used where appropriate to obtain the single channel conductance (g). In some cases, use of Ohms' law in this way is not appropriate because the I/V relation is curvilinear. When a mean g is reported, each of several patches was analysed individually; data from separate patches were not lumped. Means are given with the standard deviation (S.D.) and sample size (unless noted, the latter refers to the number of different patches).

II.42 P(O) index vs Pressure

To study the effects of varying pressures on channel activity, a series of increasing pressures were applied in a stepwise fashion. The pressure was not returned to 0 mm Hg between successive steps. The duration of each pressure step was approximately 1 minute. The probability of the SAK channel being open was defined as the time-averaged current over the single channel current. P(O) was determined as an index rather than as a true probability (whose value would vary between 0 and 1) because the absolute number of channels in the patch could not be known with certitude. The largest value of the P(O) index should then give a minimum estimate of the number of channels in the patch. The recorded data were filtered at 2 KHz (Bessel filtered) and digitized at 0.2 msec per point prior to computer analysis. When activity consisted mostly of channel currents, index P(O) was determined using a cursor-driven computer routine. Computer analysis was not possible with high pressure application because of the absence of the zero current baseline (caused by the high level of channel activity). Under these conditions, index P(O) was established by a simple

method for integrating the current: a trace was made on a Gould chart recorder, and then the area between the zero-current level and the maximum current level was cut out and weighed. The weight of multiple-channels section divided by a single channel section of comparable duration yields the P(O). Three 40 s sections for each of these high pressure recordings were taken and P(O) was obtained as the average.

II.43 Effect of membrane potential and suction release on the activity of a Cl channel.

In one patch, an excellent recording of a Cl channel sensitive to stretch allowed the dermination of 1) the effect of membrane potential on the Cl channel probability of opening and closing, and 2) the effect of suction release on the channel closure.

II.43 a F(O) and F(C) vs voltage

To calculate the probability of opening, F(O) (note that this is a different function from the P(O), the probability of being open), and of closing, F(C) of the

Cepaea Cl channel as a function of voltage (fig. 21), currents were printed on a Gould chart recorder. For each voltage, the F(O) was dermined as the total number of openings over the total number of times suction was applied. To be considered as a stretch-related opening, the channel had to open within 1 second following application of suction. Once the channel was opened with suction, F(C) was calculated as the total number of channel closings over the total number of suction releases. Here, the closing had to occur within 1 second following release of suction.

II.43 b P(O,t) vs Time

To determine the effect of suction release on the time-course of channel closure (fig.19), the conditional probability, P(O,t), of the Cl channel being open at time t after suction release was determined. To calculate P(O,t), only one Cl channel was considered. Other conductances states occured in the recording but these events were considered too rare to be included in P(O,t) dermination. The open state (130 pS; fig. 19 and 20) and the closed state were

assigned the values 1 and 0 respectively; this parameter is termed i. The total number of suction releases, N, was 22. At each time point, t, the conditional probability of the channel to be open, P(O,t), was calculated as $(\Sigma i(t)/N)$.

II.5 Kinetic analysis

Channel proteins exist in several conformational states (Hille, 1984). As mentioned before, in some states the protein forms an open pore through the membrane. In others the pore is not continuous; the channel is closed. The opening and closing of a channel is studied by measuring the time a channel conducts or not, that is, the lifetimes of channels in their open and closed states. These lifetimes (or mean times) are characterized by an exponential decay constant, T. In addition to indicating the minimum number of conformational states, kinetic analysis of open and closed states can indicate the extent to which factors (i.e. voltage, chemicals, pressure) can influence transitions among states. In order to obtain time constants, single channel recordings were

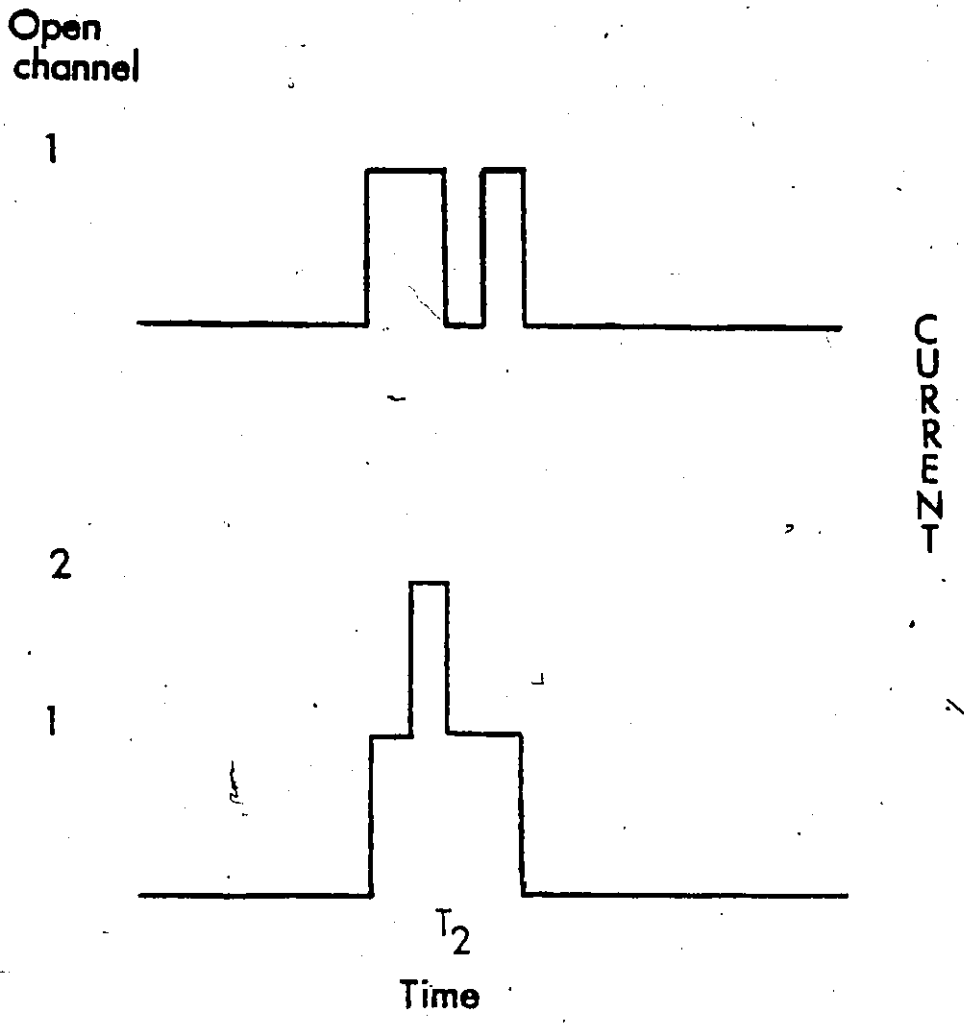
digitized, idealized records were made, and lifetime of each idealized event was determined. A histogram for each condition - open or closed - was built and exponential distribution curves were fit to these histograms in order to extract the time constants.

a) Construction of idealized record

The recording used for kinetic analysis was from a patch which originally contained more than one channel (4 were evident at one point) but the analysis was carried out on a section in which only one channel remained active. Simultaneous openings of more than one channel would have affected the T values for open events due to the ambiguity about when an open event ends (fig.5).

After filtering at 4 KHz the data were digitized at 0.025 msec per point. This digitizing rate seemed reasonable considering the excellent signal-to-noise ratio of the record under analysis.

Fig. 5 **Effect of multiple open channels on estimation of the time constant.** In the example, with two open channels, the open time (T2) is measured as being longer than with one open channel, falsifying the real lifetime of the open state of one channel. This would change the histogram distribution, thereby affecting the T value extracted from the fit to the histogram.


Open channel
1
2
1
CURRENT
T_2
Time

b) Histograms

Threshold criteria for selecting the closing and opening events (i.e. distinghishing them from background noise, and rejecting any small-channel events that might occur in the data) was established at 50% of the unit single channel current. The channel was considered to be closed when current fell below 50% of unit current amplitude for 2 or more digitized points and open when it exceeded this value. Events shorter than 0.025 msec were ignored. For the curve-fitting procedures the histogram bin widths were ajusted to contain at least 5 counts (Guharay and Sachs, 1984).

c) Time constants extraction

Time constants were extracted from the data by regression of a sum of exponential probability density functions (Corey, 1983). The time distribution is predicted by the following equation:

$$\#\ \text{with time}(t, dt) = N\ k\ \exp(-kt)dt + N\ k\ \exp(-kt)dt + \ldots \qquad (1)$$

N= number of events

k= rate constant (second)

t=time (second)

In essence, for a given sum of exponentials, various values for numbers of N values and k were tried to find the values which best represented the distribution . The routine used was a weighted Marquardt non-linear regression (Sigurdson et al., 1987).. The number of exponential terms to best fit the data was chosen on the basis of a minimum Chi-square value. Time constants were obtained by calculation of the inverse of the rate constant, T= 1/k.

II.6 Equations for studying channel selectivity

One of the techniques used to establish the ionic selectivity of a channel is to compare the channel's reversal potential to equilibrium potentials of the ions which might be permeant. The equilibrium potential is determined by the electrochemical driving forces acting on the ion. At this zero-current potential (when no inward or outward current flows),

the potential is said to reverse. When the channel is permeable to one ion, the system can equilibrate; the equilibrium potential is predicted by the Nernst equation. The Goldman-Hodgkin-Katz equation predicts the steady-state potential when the channel is permeable to more than one ion (Hille, 1984), provided ion concentrations are kept constant.

II.61 Nernst equation

The channel is permeable to one ion.

$$Vr = \frac{RT}{FZ} \ln \frac{[ion]}{[ion]} \qquad (2)$$

where Vr is the reversal potential (volts), R is the gas constant (8.31 J/mole °K); T is the temperature (Kelvin), F is the Faraday constant (9.65 X 10^4 C/mole), Z is the ion valency and [ion] are the ion concentrations outside and inside (mole/l).

II.62 Goldman-Hodgkin-Katz (GHK) current equation (Pk calculation)

The Nernst equation is inappropriate to calculate the reversal potential of a channel permeable to more than one ion or to predict the current flow for various values of membranes voltages. Instead, one uses the GHK equation. A form of the GHK equation that is applicable for predicting currents when there is only one ion available for (or capable of) permeation is

$$I = (P)\frac{(VF)}{(RT)}\frac{([K]_{out} - [K]_{in}\exp(VF/RT))}{1-\exp(VF/RT)} \qquad (3)$$

This non-linear equation simplifies to a linear one - Ohm's law - when external and internal ion concentrations are equal. In particular, when K concentrations on both sides of the membrane are equal and there are no other ions, the equation becomes

$$I = (P)(VF)([K])/(RT) \qquad (4)$$

where P is the permeability to K of a single channel in cm/sec., V is the membrane potential (volts), K is

the K concentration in mol/cm^3, F, R and T are the same constant as in the Nernst equation (Benham, Bolton, Lang and Takewaki, 1986). In essence, since I=Vg (Ohm's law; g=conductance), the terms on the right hand side of equation 4, other than V, are the factors which determine the single channel conductance for these simple conditions. A value for the permeability constant can be determined experimentally by solving for P when I/V relations are obtained under these conditions. It is advisable to generate the entire I/V relation rather than assume that any one I, V pair falls on a linear region of the I/V relation.

CHAPITRE III

III RESULTS

III.1 SAK channel

III.11 SAK channel characterization

In cell-attached recordings with NCS in the pipette, stretch-sensitive outward currents were observed in virtually all patches when the membrane was depolarized from rest (fig.6). Stretch-activated currents were not seen at the resting potential or when the membrane was hyperpolarized. The I/V relation obtained for the stretch-activated events under these physiological conditions is approximately linear over the voltage range -40 to 40 mV, giving a channel conductance of 58 ± 3.7 pS (Standard deviation, S.D., N=5) (fig.7). Inward currents could not be detected, but curvilinear extrapolation of the foot of the curve suggests a zero-current potential in the region of the resting potential of Cepaea neurons ($V_r \simeq$ -50 mV; Trams et al., 1965). Under physiological conditions, the only ions whose equilibrium potential would be near the resting potential are K and Cl. To determine which of these ions was responsible for the stretch-activated

Fig. 6. Typical *Cepaea* SAK channel recordings in cell-attached condition with NCS and 70 mM K in the pipette. With NCS, no inward current is seen due to low K concentration (4 mM) in the pipette. With 70 mM K, inward current as well as outward currents are observed due to high K concentration in the pipette and in cytoplasm, respectively. Applied pressure, -70 mm Hg. Filter, 2 KHz.

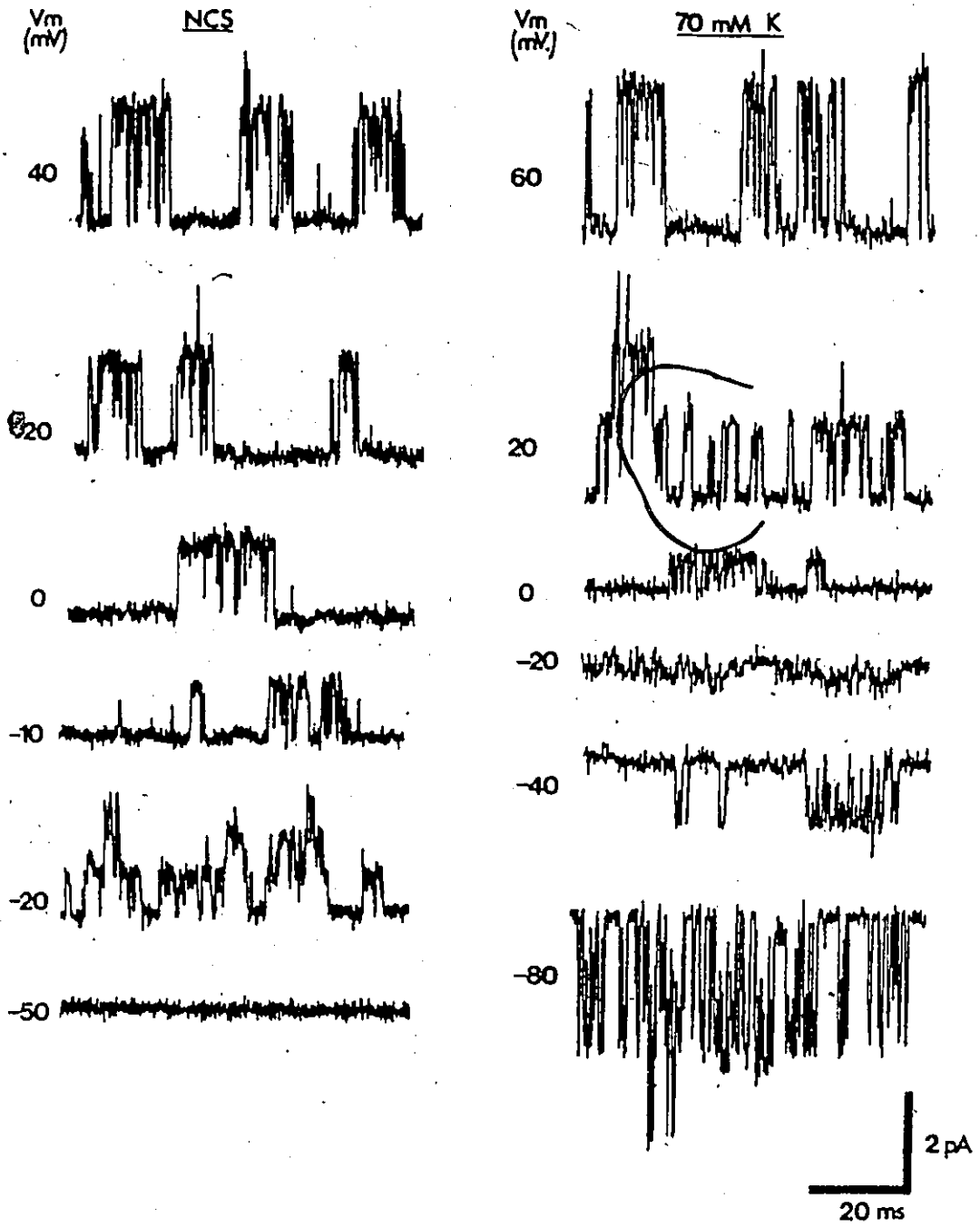
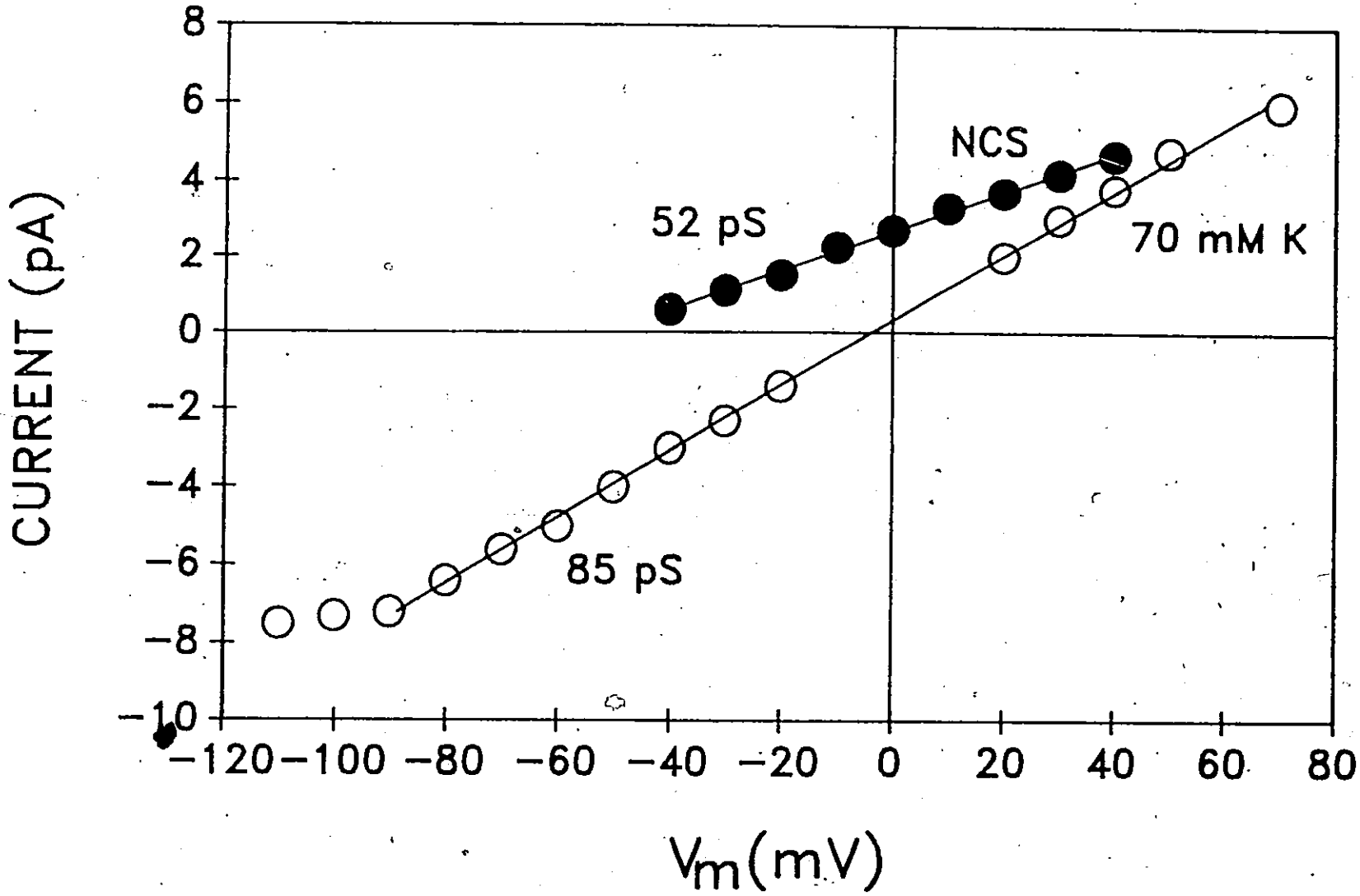

Vm (mV)
NCS
40
20
0
-10
-20
-50
Vm (mV)
70 mM K
60
20
0
-20
-40
-80
2 pA
20 ms

Fig. 7 **Examples of current-voltage relations in cell-attached condition for the *Cepaea* SAK channel.** O) Pipette solution, NCS. ●) Pipette solution, 70 mM K. There is a shift in the depolarizing direction with higher K concentration.


8
6
4
2
0
−2
−4
−6
−8
−10
CURRENT (pA)
NCS
52 pS
70 mM K
85 pS
−120 −100 −80 −60 −40 −20 0 20 40 60 80
Vm(mV)

currents, the K concentration in the pipette was increased from 4 to 70 mM while maintaining the Cl concentration constant (Table 1). This substitution caused a shift of the I/V curve in the depolarizing direction, indicating that the stretch-activated channel is K rather than Cl permeable (fig.6 and 7). The channel conductance under these conditions was 76 ± 13 pS (S.D., N=8). Substitution of 70 mM K with potassium salts of acetate or gluconate saline (anions which are not permeant through most Cl channels; Welsh, 1986) did not abolish the stretch-activated currents. We can conclude that the stretch-sensitive channel observed in Cepaea neurons under cell-attached conditions is, like that in Lymnaea heart cells (Sigurdson et al., 1987) and neurons (Sigurdson, Bédard and Morris, 1987), a stretch-activated K (SAK) channel.

To further characterize the permeation properties of Cepaea SAK channel, the channels were studied in inside-out patches under a variety of ionic conditions.

With NCS in the pipette and in the bath, outward SAK channel currents are readily apparent in the cell-attached configuration, but no currents can be elicited

once an inside-out patch is formed. This disappearance is the result not of excision process, but of insufficient permeant ions; with 70 mM K in the pipette and NCS in the bath, SAK channel currents occur in both the cell-attached and excised condition. The non-linear I/V curves (N=4) obtained from excised patches under these conditions (70 mM K in pipette, NCS in the bath) extrapolate to a zero-current potential at about +70 mV (N=4), very close to the Nernst equation prediction (+72 mV), confirming that the channel is highly selective for K over Na and Cl (fig. 9; the equilibrium potentials for Na and Cl would be -44 and 0 mV respectively).

Like other K channels, the SAK channel shows deviations from independence (French and Wells, 1977; Latorre and Miller, 1983; Begham et al., 1986; Ashcroft, Kakei, Kelly and Sutton, 1987). The principle of independence states that any ion crossing the membrane is independent of the other ions and does not interact with channel structures which offer resistance to ion movements (Hille, 1984). Deviations are shown in figure 9 where the experimental data are compared to the expectations of the Goldman, Hodgkin,

Katz (GHK) equation in which transit of the ions is dependent only on the electrochemical driving force (Hille, 1984).

A permeability constant, Pk, of 3.4 X 10^{-13} cm/s was determined using the experimentally observed limiting conductance (hyperpolarizing quadrant) obtained under conditions of symmetrical 70 mM K (see method for Pk calculation). As in the case with a variety of K channels, non-linearities appear in the depolarizing quadrant (French et Wells., 1977; Marty, 1983; Ashcroft et al., 1987).

Figure 9 shows that the GHK I/V relation (given this Pk) for 70 mM K* out/4 mM K in (NCS) accurately predicts the experimental values for inward current when the major "intracellular" cation is choline (N=4; Table 1; Na channel are impermeant to choline; Coronado and Miller, 1982). But when Na is used instead of choline, the I/V curve tends toward the predicted values only at its extremes, (i.e. near the reversal potential, 72 mV, and as the limiting conductance, 92 ± 3 pS , N=3 patches, is approached.). At intermediate voltages, inward K currents are smaller when choline is

Fig.8 **Substitution of internal Na by choline caused an increase in the amplitude of the SAK channel inward currents.** Examples of single-channel currents recorded from inside-out patches with 70 mM K^+ in the pipette and with a) 0 mM Na (80 mM choline Cl) in the bath and b) 80 mM Na in the bath (NCS). Applied pressure, -55 mm Hg; filtre, 2KHz.

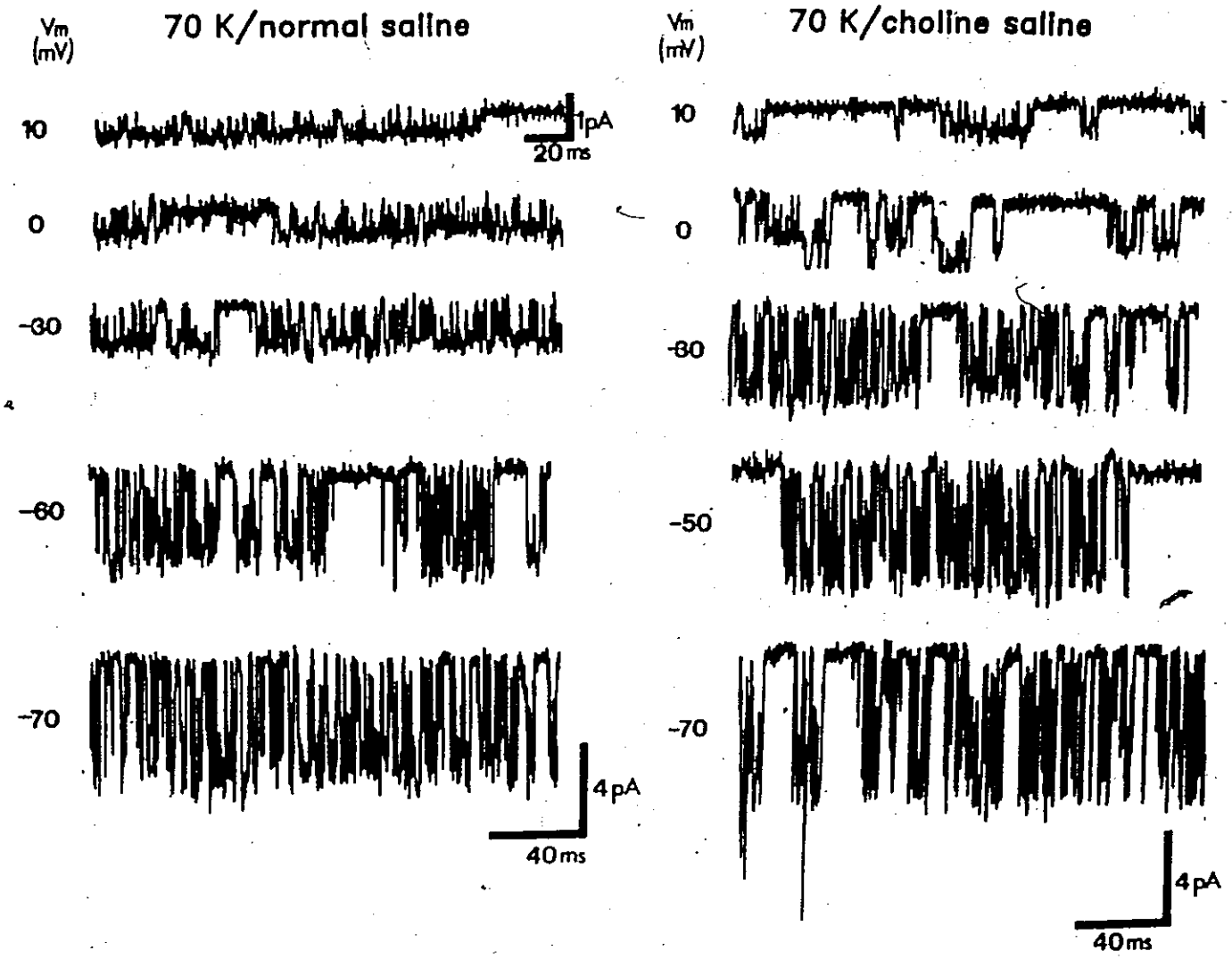
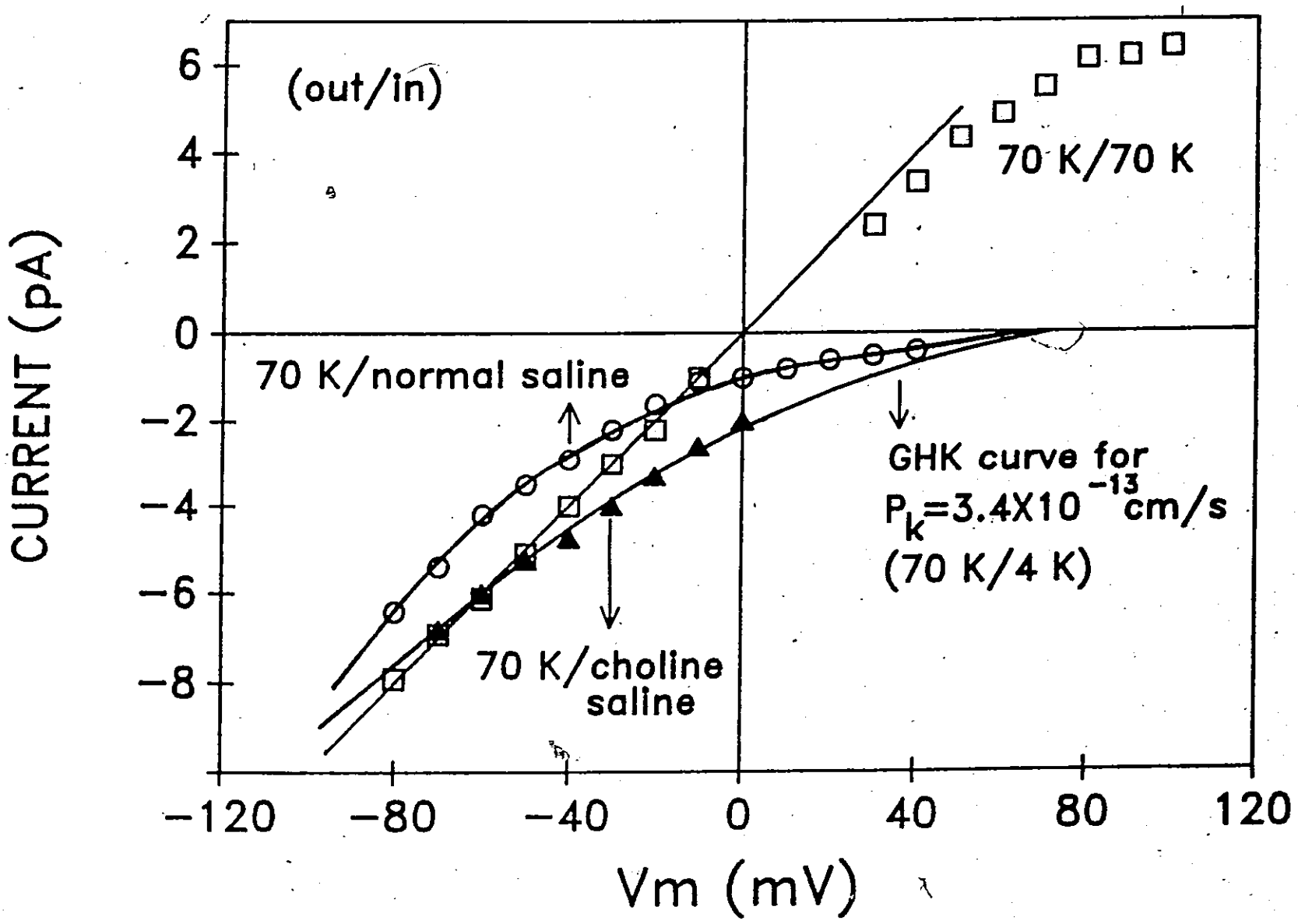

Vm (mV)
70 K/normal saline
10
0
-30
-60
-70
1pA
20ms
4pA
40ms
Vm (mV)
70 K/choline saline
10
0
-30
-50
-70
4pA
40ms

Fig. 9 A GHK analysis of currents through the SAK channel. □) 70 mM K* on both sides of the membrane; Vr, 0mV; straight line corresponds to a conductance of 92 pS. O) Experimental curve (fit by eye) with 70 mM K* in the pipette and 80 mM Na (NCS) in the bath; Vr is near 70 mV. ▲) Data obtained with 70 mM K* in the pipette and 0 mM Na (80 mM choline) surimposed to the predicted GHK curve in the same experimental condition (Pk used to generate the curve from equation 3 is 3.4 X 10^{-13} cm/s); predicted Vr is 72 mV. At high hyperpolarization, the curves with and without Na at the inner face of the membrane approach each other.


(out/in)
70 K/70 K
70 K/normal saline
GHK curve for
P_k=3.4X10^{-13}cm/s
(70 K/4 K)
70 K/choline
saline
CURRENT (pA)
Vm (mV)
6
4
2
0
−2
−4
−6
−8
−120
−80
−40
0
40
80
120

substituted for Na (fig. 8 and 9). A plausible explanation is that the SAK channel has a site in the permeation pathway which is capable of being blocked from the intracellular side by (non-permeant) Na ions. This explanation is consistent with the observation that the block is relieved by hyperpolarization suggesting a voltage-dependent block (hyperpolarization should decrease the electrical force driving Na ions into the blocking site) and with the observation that the reversal potential is apparently unaffected by Na (a non-permeation blocking ion would not change the reversal potential, only the magnitude of the current). Block by internal Na has been reported in Ca-activated-K-channel (Marty, 1983; Yellen, 1984). The block observed here is also similar to internal TEA block which interfer with inward K current in squid giant axon (French and Wells., 1977).

Of the cations normally available to it, the SAK channel is permeable exclusively to K. With high Ca (10^{-1} M; N=8), Mg (5 X 10^{-1} M; N=4) or Na (84 mM; N=5) solutions in the pipette and NCS in the bath, stretch-activated currents could not be detected in excised patches at any voltages. Absence of SAK channel activity was not due to artefact such as vesicle

formation since large Cl channels were observed in some patches. Thus, though Na, Mg and/or Ca may be able to block or interfer with K permeation (perhaps accounting for the low conductance of the cell-attached, normal saline recordings), they are not themselves permeant. No experiments were done in symmetrical 0 mM K so the possibility remains that (by analogy with the 2-sites Ca channel; Tsien, Hess, McCleskey and Rosenberg, 1987) the channel becomes less selective in the absence of its favoured ion. It seems evident, however, that in the presence of 4 mM or more intracellular K, no other physiologically-relevant cations can use the channel.

The SAK channel shows a further departure from independence that, once again, is characteristic of K channels (Hille, 1984): its conductance is not the linearly increasing function of K concentration that would be predicted from, for example, equation 3. Cell-attached recordings with 4, 40, 70 and 150 mM K (Table 1) in the pipette gave conductances of 0 (or at least undectable), 71 ± 10 pS (S.D., N=6), 76 ± 10 pS (S.D., N=8) and 81 ± 5 pS (S.D., N=5) respectively. The last three values are not significantly different; in effect, the conductance has already saturated at 40

mM.

The Cepaea SAK channel is essentially insensitive to the presence or absence of Ca on the external and internal face. The channel activity did not increase or decrease with 10^{-7} M Ca (NCS) or with Ca-free solutions (N=5) (Table 1). Contrary to a K channel present in mammalian neurons which is modulated by internal Na (Dryer, Fujii and Martin, 1988), SAK channel activation was revealed to be insensitive to Na ion. When inside-out patches were performed, the SAK channel activity was not affected by 80 mM Na (NCS) on the outside or inside of the membrane. On all of 10 patches examined the SAK channel activity tended to increase at very high potentials, (more than +120 mV) but this was not characterized further, since it is unlikely to have any physiological significance. Specific K channel blockers such as external TEA (10^{-2} M; N=5) (Coronado et al., 1982; Quandt, 1988), and 4-AP (10^{-3} M; N=4) (Meves and Pichon, 1977; Thompson, 1977; Quandt, 1988) did not affect SAK channel activity. Quinidine (Iwatsuki and Petersen, 1985) at 10^{-4} M (N=5) did not have any obvious effect but at 10^{-3} M (N=5) it increases the flickeriness of the currents and reduces

the channel conductance (fig.10). Similar effects of quinidine and a lack of effect from TEA and 4-AP are also observed for Lymnaea SAK channels (Brezden et al, 1986).

III.2 SAK channel sensitivity to pressure

The SAK channel activity in Cepaea was usually absent when no suction was applied (90% of the patches). This suggests that in Cepaea neurons there could be only a very minor contribution of the SAK channel to the resting potential, unless the membrane experiences chronic tension in vivo.

Application of suction did not alter the channel conductance (fig.11). When negative pressure was applied to the patch pipette the probability of the channel being open, P(O), was increased (fig.12). The elevated P(O) was maintained as long as the pressure was sustained. The release of suction caused, within a second, a decrease in the channel activity to near the basal level of activity. At low and saturating pressures the number of open channels was more constant

Fig.10 The effect of quinidine effect on SAK channel currents. Typical single-channel currents recorded at 40 mV in cell-attached condition with 70 mM K and two quinidine concentrations in the pipette. There is no obvious effect except a small increase in the flickeriness at 10^{-4} M but at 10^{-3} M, this flickery block leads to an apparent decrease of the channel current due to the limited temporal resolution. Applied pressure, -60 mm Hg; Filter, 2 KHz.

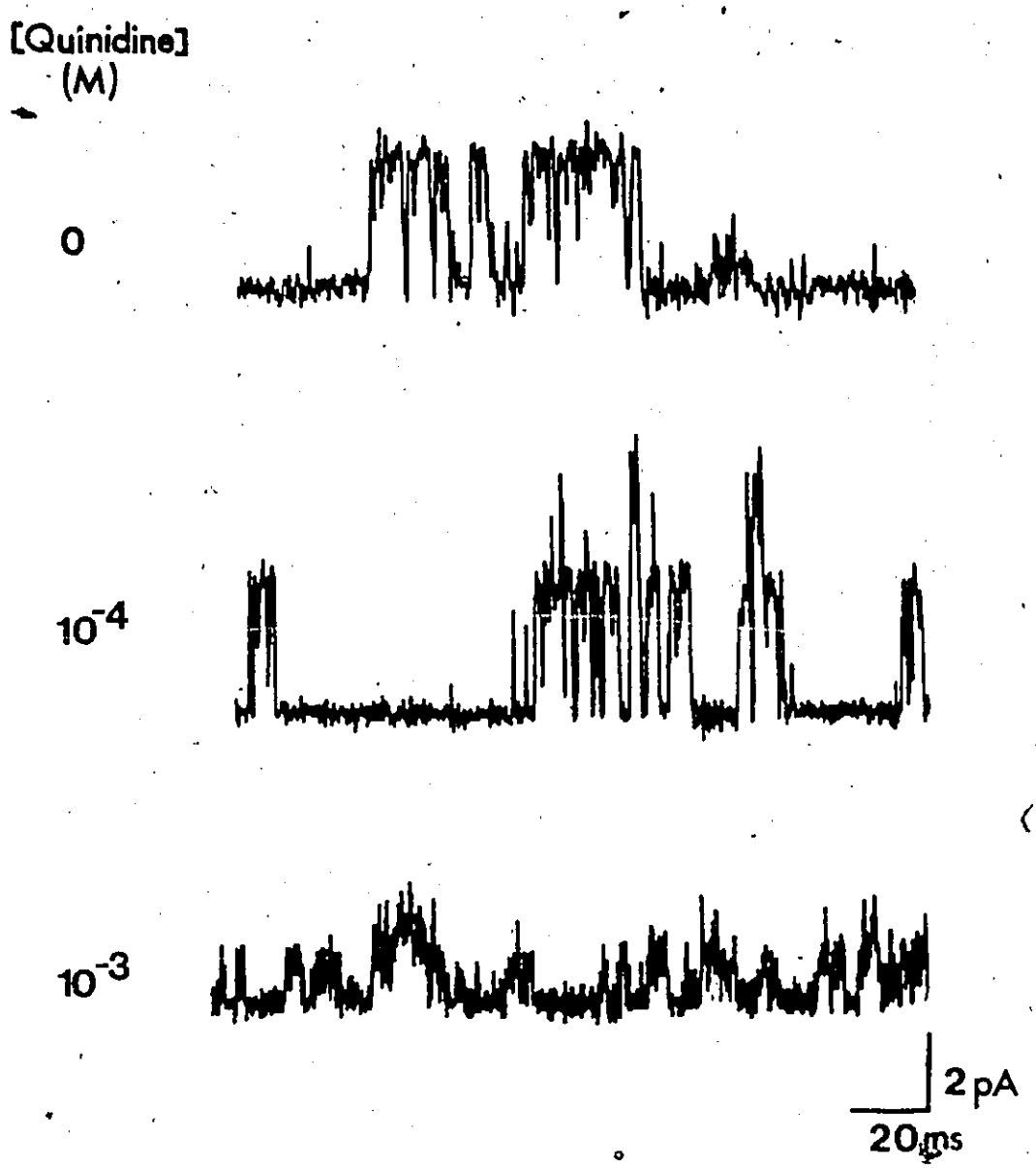
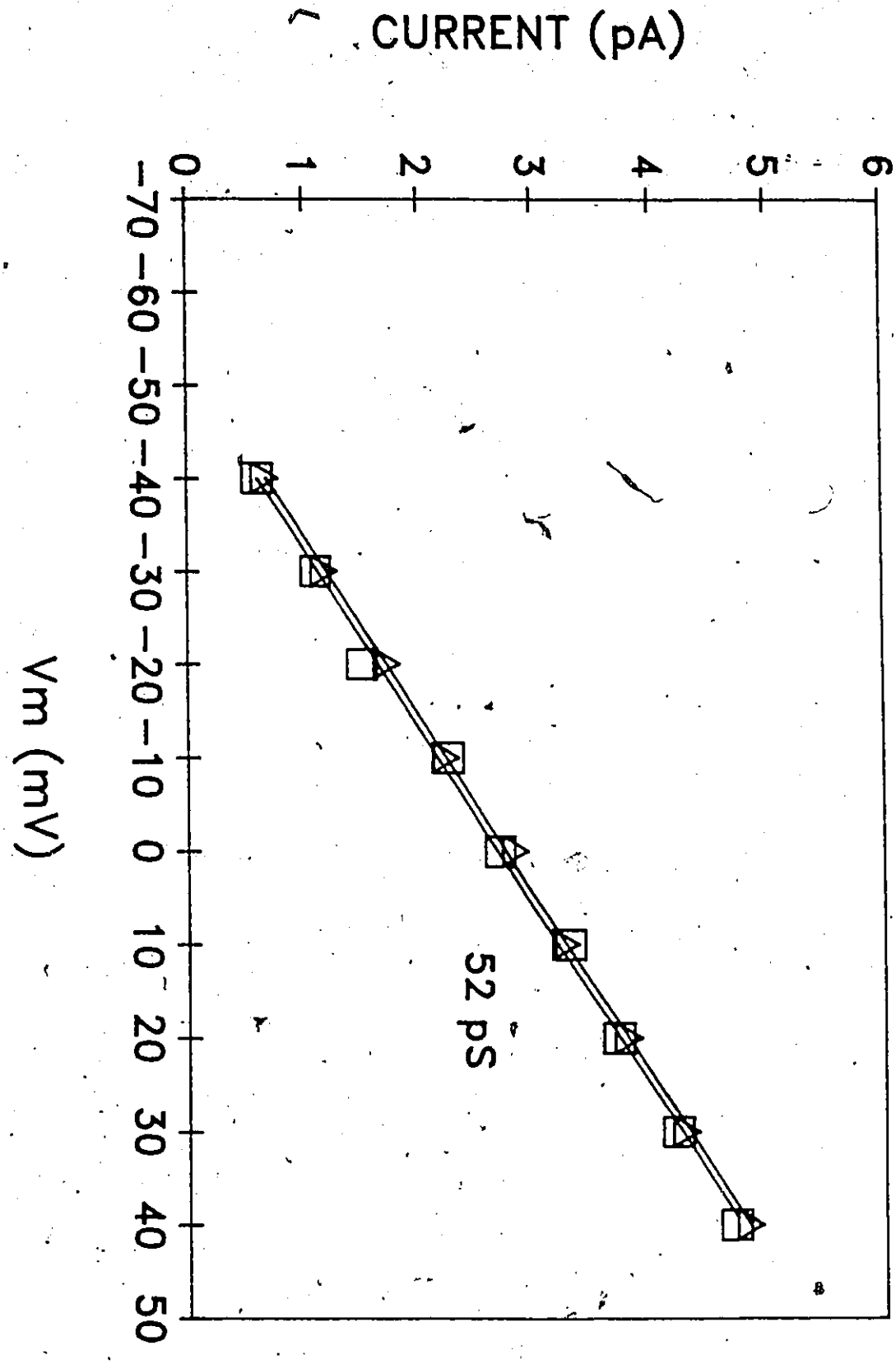

[Quinidine]
(M)
0
10^{-4}
10^{-3}
2 pA
20 ms

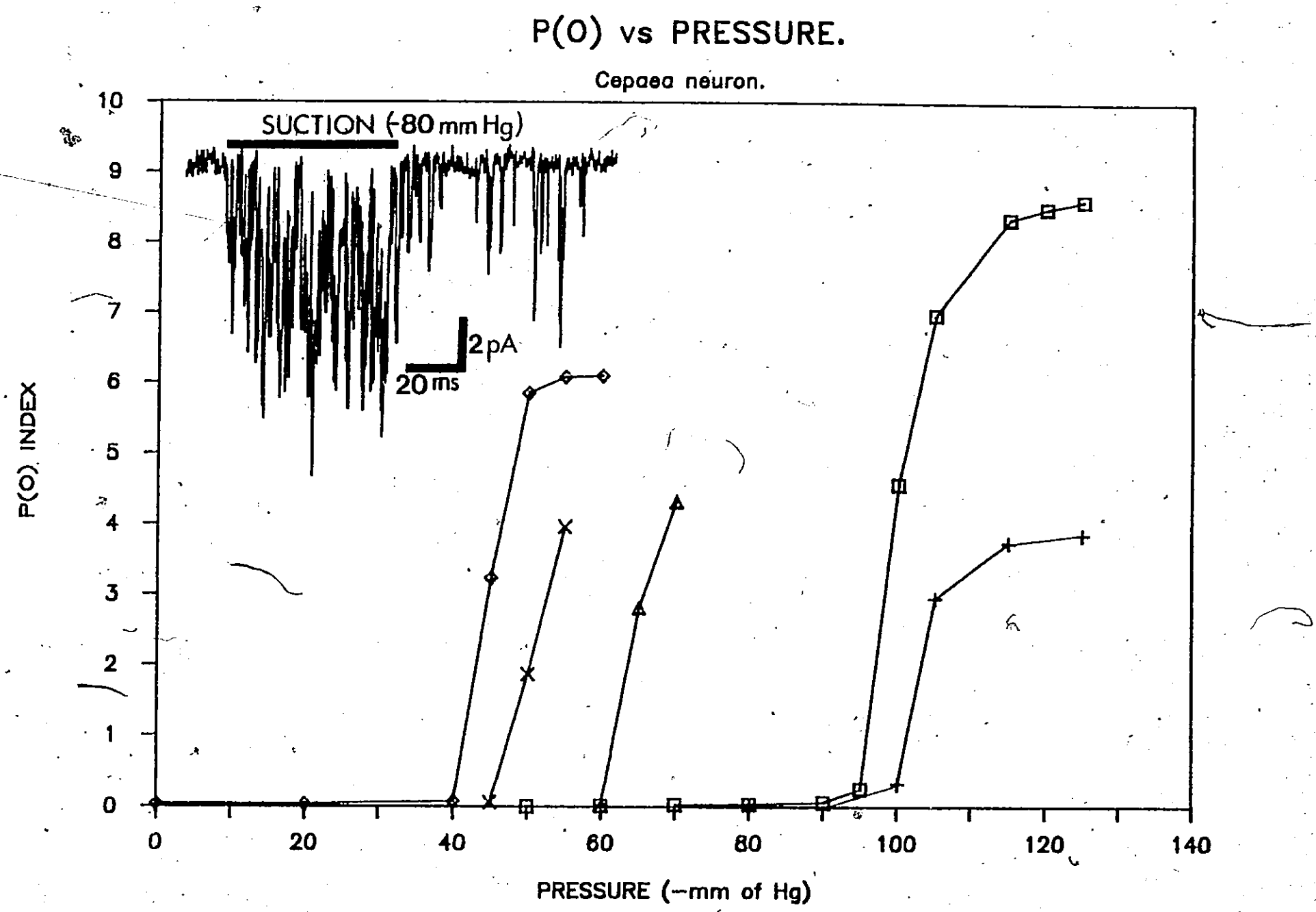
Fig.11 Pressure does not cause an increase in SAK channel conductance. Typical current-voltage relations in cell-attached condition (NCS in the pipette) with applied pressure (-60 mm Hg; upper line, Δ) and without pressure (lower line, □).


CURRENT (pA)
0
1
2
3
4
5
6
−70
−60
−50
−40
−30
−20
−10
0
10
20
30
40
50
Vm (mV)
52 pS

than at intermediate pressures (steeper part of the slope, fig.12). Positive pressures increased the channel activity but the loss of patches because of membrane rupture at high pressures often prevented the determination of P(O) up to saturation. At maximum pressure 7-8 channels were usually seen per patch. Considering an approximate pipette tip area of 6-4 µm (based on calculations of the membrane area associated with patches made with pipettes of 10-13 MOhms; Sakamann and Neher, 1983), the SAK channel density is 1-2 channels per µm.

The P(O) index vs negative pressure relation is approximately sigmoidal (fig.12) as had been shown for other SA channel (Guharay and Sachs, 1984; Cooper et al., 1986; Sigurdson et al., 1987). Even when the full P(O) curve could not be obtained (because the patch did not last long enough), it was possible to get a reasonable estimate of the half-maximum pressure, because the rise of the curve is so steep. In fact, since the usual procedure upon obtaining a patch was to monitor the transducer output while rapidly applying suction up to the range where activation was noted, it was possible to build up an overall picture of the stretch-sensitivity of the SAK channel even when no

Fig. 12 **Negative pressure caused an increase in SAK channel probability of being open.** Cell-attached; pipette saline, 70 mM K; Vm, 0 mV. Different symbols denote different patches. Examples of group I (the three left most curves) and group II (the two curves to the right) classes of sensitivity are given. Inset: Cell-attached; pipette saline, 70 mM K; Vm, -40 mV; filter, 2 KHz.


P(O) vs PRESSURE.
Cepaea neuron.
SUCTION (-80 mm Hg)
2 pA
20 ms
P(O) INDEX
PRESSURE (−mm of Hg)
0
1
2
3
4
5
6
7
8
9
10
0
20
40
60
80
100
120
140

data was formally recorded. Most patches showed full activation well below -70 mm Hg (group I; fig.12). About 20% of the patches (group II), however, had more recalcitrant SAK channels. Values from curves of the group I class of patches had half-saturation values of -55 ± 10 mm Hg (N=5 patches). For the group II patches from which quantification was difficult (because of the increased danger of patch rupture) half-saturation occured at -100 ± 5 mm Hg (N=4). This decreased sensitivity was correlated with cases where seal formation was spontaneous (when the pipette is put on the cell membrane, gigaseal formation usually requires gentle transient suction, but sometimes it can occur without suction, i.e. "spontaneously"), whereas higher stretch sensitivity was observed when a seal formed following application of suction. Membrane invagination in the pipette in the latter case could rendered membrane deformation or stretching easier when suction is applied.

III.3 Kinetic analysis

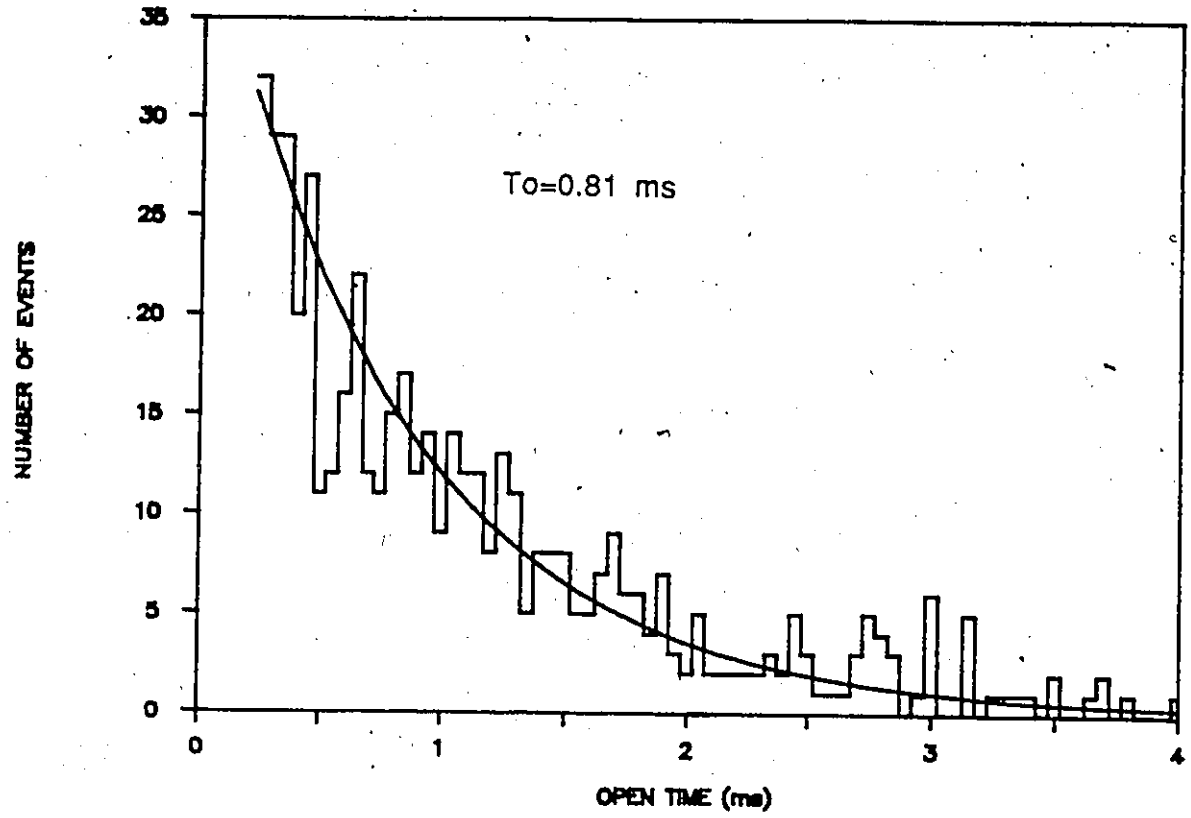
Patches in which the recording characteristics of SA channels are suitable for detailed kinetic analysis are

rare. One such patch was obtained and the opportunity was used to make a comparison with the Lymnaea SAK channel. The analysis was undertaken at 2 different pressures, 0 and -90 mm Hg which was close to the threshold for activation in this patch. The patch was lost when more intense suction was applied in an attempt to study the kinetics at more pressure points. The cell-attached patch was held at 0 mV, with KCS in the pipette. It should be noted that the number of events at 0 mm Hg is fewer than at -90 mm Hg due to the brief duration of the recording.

The distribution of open times at 0 and -90 mm Hg is represented by single exponentials (fig.13 and 14). For the closed time distributions, three exponentials give the best fit at both pressures as illustrated in figure 13 and 14. Thus, the SAK channel has a minimum of four kinetic states; one open and three closed. Application of pressure does not apparently create new kinetic states.

Comparison of the time constants for the closed time distributions (fig. 13 and 14) indicates that there is a sharp decrease in the longest closed time constant at

Fig. 13 **Open (a) and closed (b) time histogram for SAK channel at 0 mm Hg.** Cell-attached; pipette solution, 70 mM K; Vm=0 mV. The time constants associated with function (continuous line drawn over the histogram bins) are indicated (o, open; c, closed). From the 95% confidence limits associated with the fits, the error bounds on time constants To, Tc_1, Tc_2 and Tc_3 are + 0.013, 0.057, 0.093, 5.245, respectively.


To=0.81 ms
NUMBER OF EVENTS
OPEN TIME (ms)
0
5
10
15
20
25
30
35
0
1
2
3
4

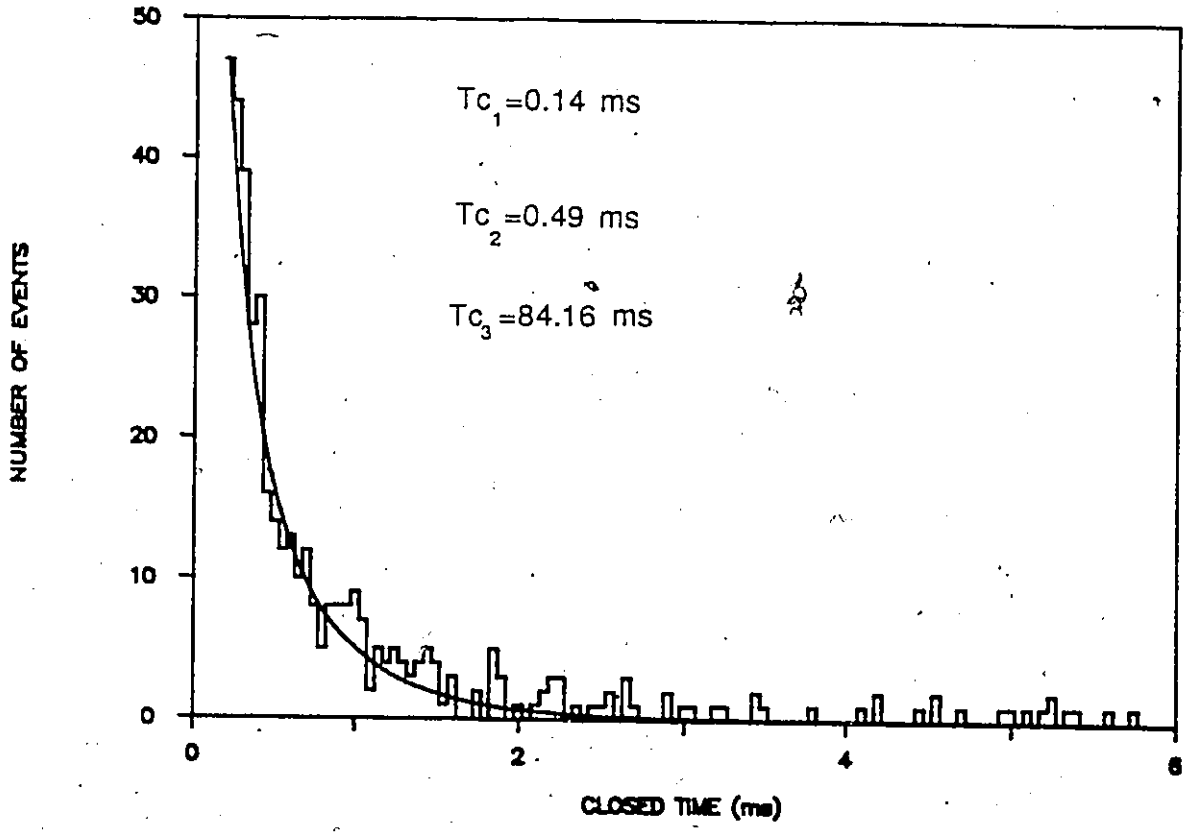
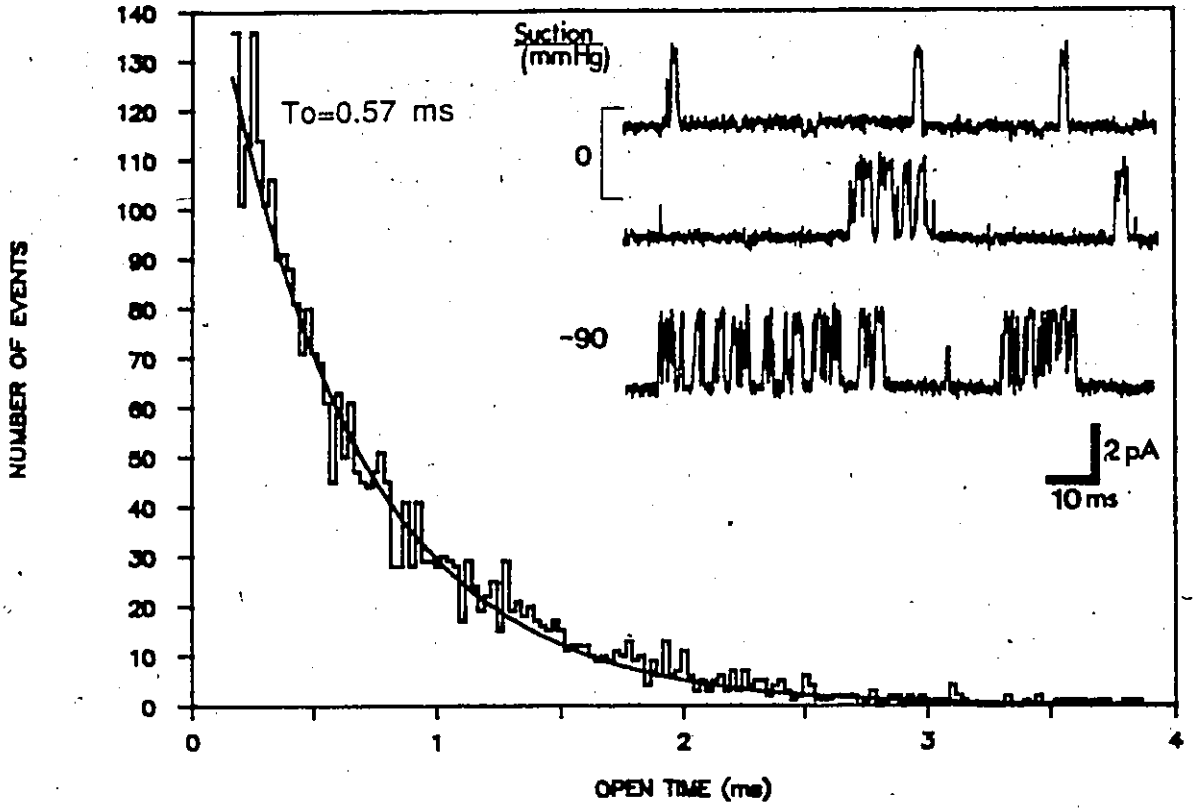
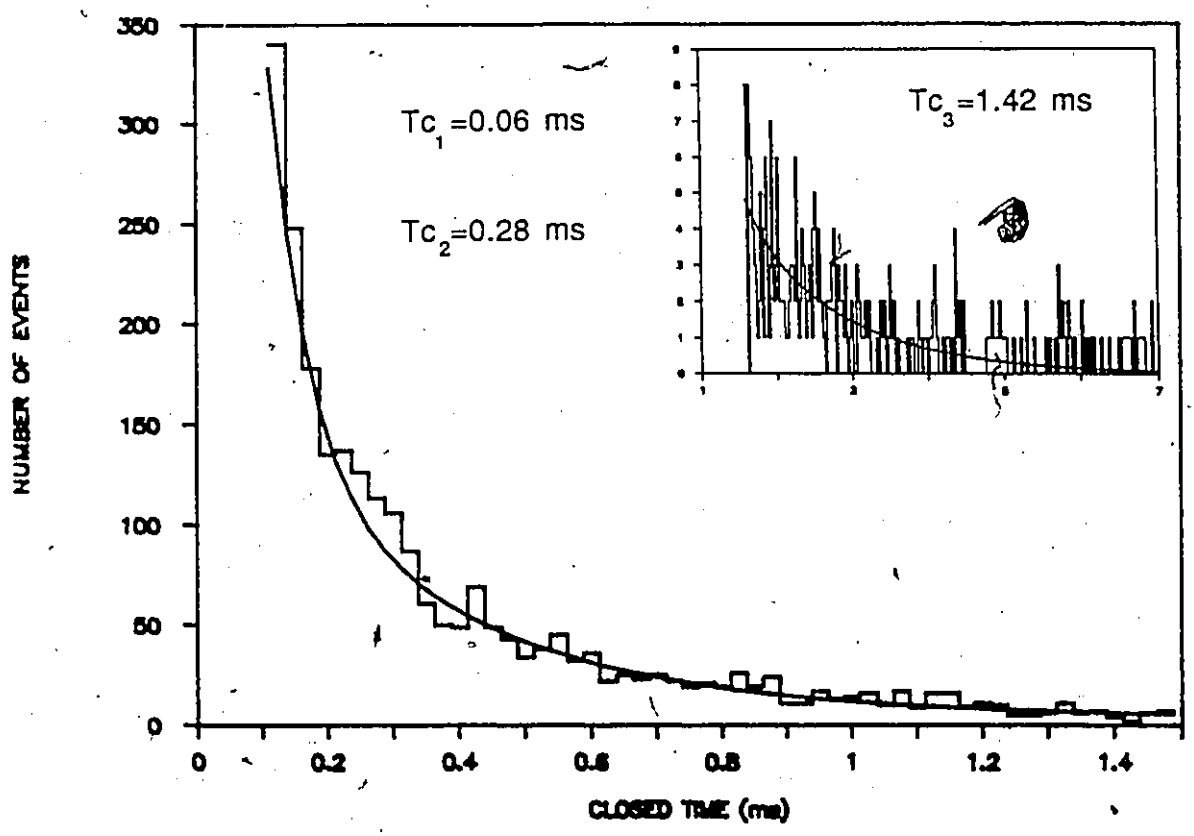

Tc1=0.14 ms
Tc2=0.49 ms
Tc3=84.16 ms
NUMBER OF EVENTS
CLOSED TIME (ms)
0
10
20
30
40
50
0
2
4
6

Fig.14 **Open (a) and closed (b) time histograms for SAK channel at -90 mm Hg.** Cell-attached; pipette solution, 70 mM K; Vm=0 mV. The time constants are indicated (o, open; c, closed). From the 95% confidence limits associated with the fits, the error bounds on time constants To, Tc_1, Tc_2 and Tc_3 are + 0.056, 0.007, 0.029, 0.038, respectively. The tail of the third exponential fitted to the closed times is shown as an inset.

a


To=0.57 ms
Suction (mmHg)
0
-90
2 pA
10 ms
NUMBER OF EVENTS
OPEN TIME (ms)

b


Tc1=0.06 ms
Tc2=0.28 ms
Tc3=1.42 ms
NUMBER OF EVENTS
CLOSED TIME (ms)

-90 mm Hg. At -90 mm Hg the value of Tc_3 is 60 times smaller than that obtained at 0 mm Hg. Thus, as in the case of the Lymnaea stagnalis SAK channel (Sigurdson et al., 1987), the longest closed state of the Cepaea SAK channel is affected by stretch.

The time constant of the open time distribution is slightly affected by suction. At -90 mm Hg, To is 1.4 times smaller than at 0 mm Hg. This could clearly not contribute to the increase in P(O) with suction. For the two faster closed time constants, increasing the pressure to -90 mm Hg produces decreases of, respectively 2.2 and 1.7 times. Since these brief closures make up such a small fraction of the overall record, the changes observed here could not account for the 10-fold increase in P(O) seen when suction is increased from 0 to -90 mm Hg. Thus, the principal contributor to the increased P(O) must be the long closed time constant.

Given that these results were obtained from a recording in which there was only ever one channel open, and given that Tc_1 and Tc_2 are small compared to Tc_3, one can assume that the brief openings of the SAK channel are followed by the opening of the same

channel. By contrast, when a channel enters its long closed state, there is a stronger probability that the next opening will be that of a different channel. In this particular case, however, the absence of double openings when the P(O) was high (at -90 mm Hg, P(O) is 0.99) suggests that only one channel was active and that even the longest closed time can be ascribed to the channel itself, rather than to a population of channels. In other words, without application of tension, this channel was entering closed periods of about one-tenth of a second. Suction decreases this long closed interval, allowing the channel to enter a "bursting" state in which it undergoes periods of activity involving excursions among its short-lived open and and closed states.

III.4 SACl channel in *Cepaea*

III.41 SACl channel characterization

In many patches when negative pressure was applied to excised patches to trigger the SAK channel opening, a large conductance channel was activated (N=10 patches). This channel was distinguished from the SAK channel on the following criteria: it was not flickery, it has a higher conductance and it shows multiple conductances (fig. 15). With normal saline in the bath (114 mM Cl) and 70 mM K* (70 mM Cl) in the pipette, large currents were seen at depolarized and hyperpolarized potentials (fig. 16 and 17). Precisely as predicted by the Nernst equation for Cl in these experimental conditions, the current/voltage relation gave a reversal potential of +12 mV. The absence of the current in Cl free solution (Cl being substituted by organic ions impermeant to the Cl channel such as gluconate or acetate) suggests that the observed current is due to Cl. Similar large conductance Cl channel have been observed in Lymnaea stagnalis neurons (Geletyuk and Kazachenko, 1985) and in alveolar epithelial cells (Krouse, Scheinder and Gage, 1986). The channel was never observed in the

Fig.15 Comparison between the Cepaea SAC1 and SAK channel. Inside-out patches pipette solution, 70 mM K; bath solution, NCS; Vm, -20 mV; initial filter, 2 KHz; applied pressure, -35 mm Hg (SAC1) and -55 mm Hg (SAK). The channels are from different patches. Dotted lines indicates the zero-current level.

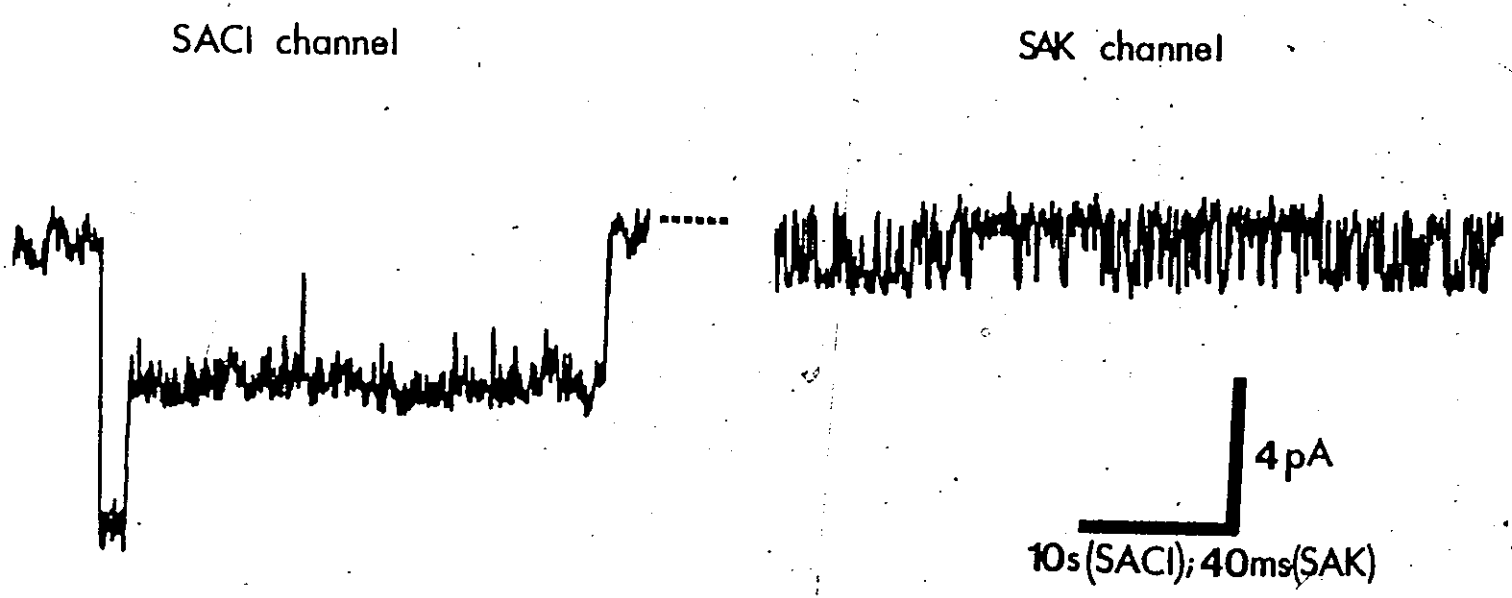
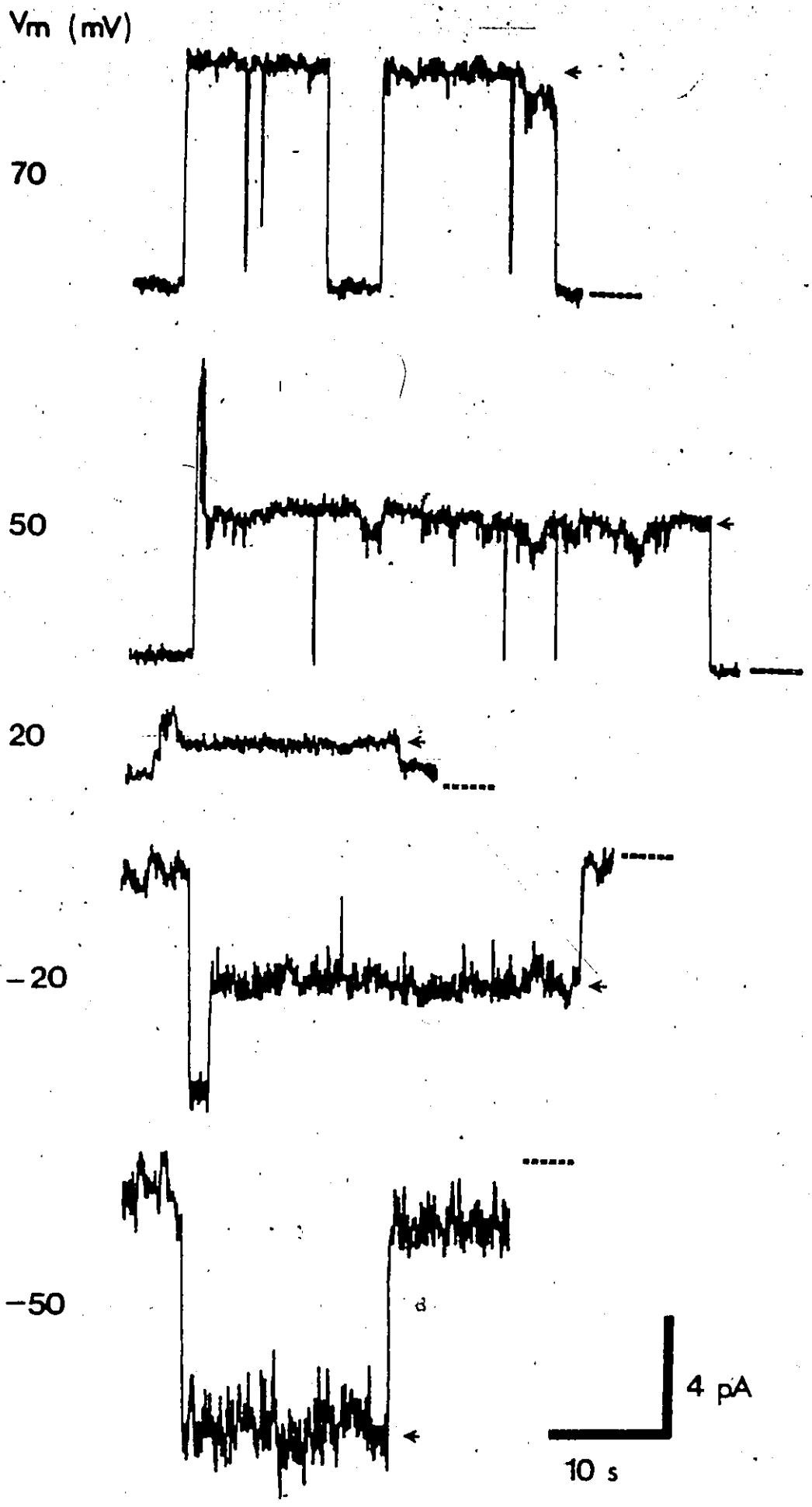

SACI channel
SAK channel
4 pA
10s (SACI); 40ms (SAK)

Fig.16. Cl channel sensitive to stretch in *Cepaea nemoralis* neurons. Examples of SACl single-channel currents from an inside-out patch; pipette solution, 70 mM K*; bath solution, NCS. Applied pressure, -35 mm Hg; filter, 2 KHz; arrows denote the modal state.


Vm (mV)
70
50
20
−20
−50
4 pA
10 s

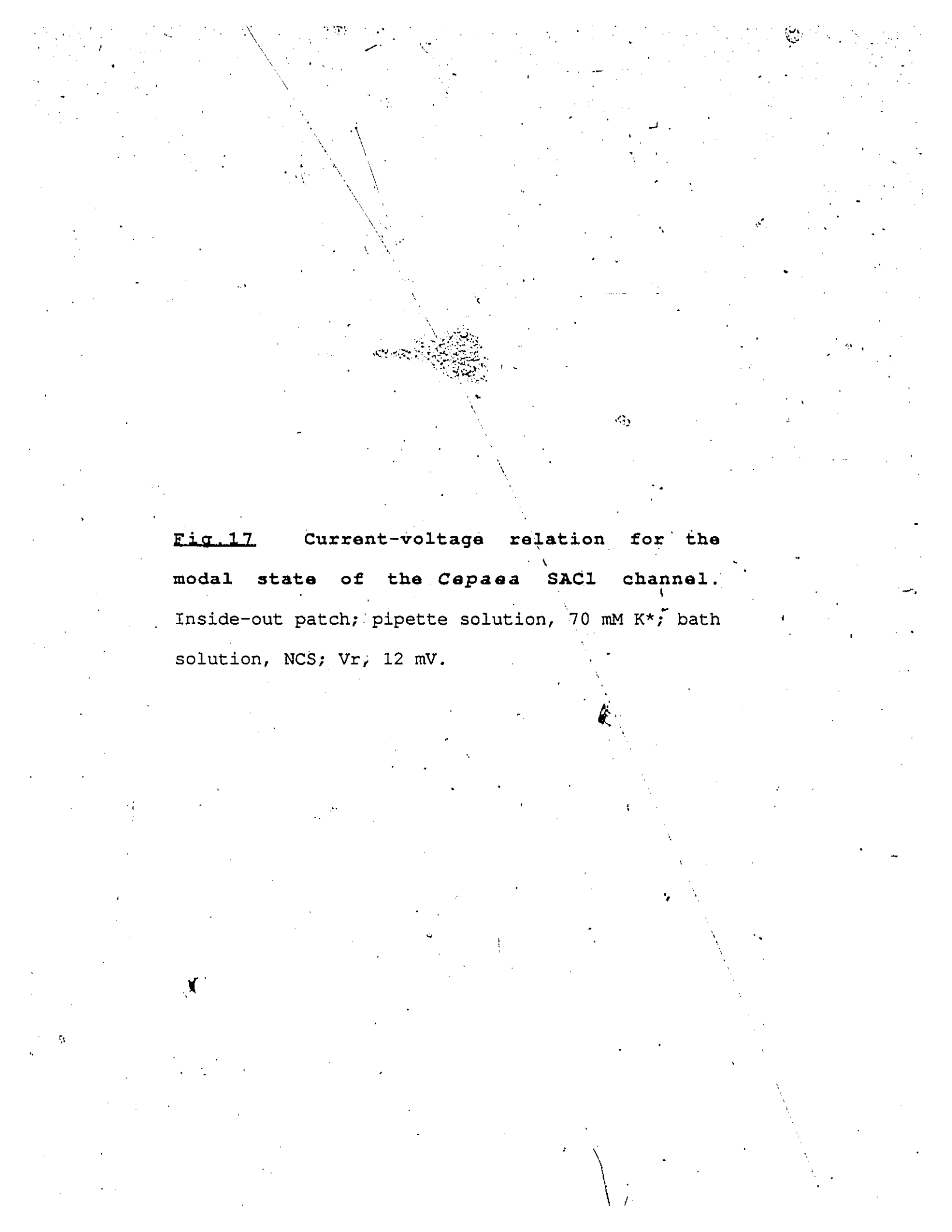
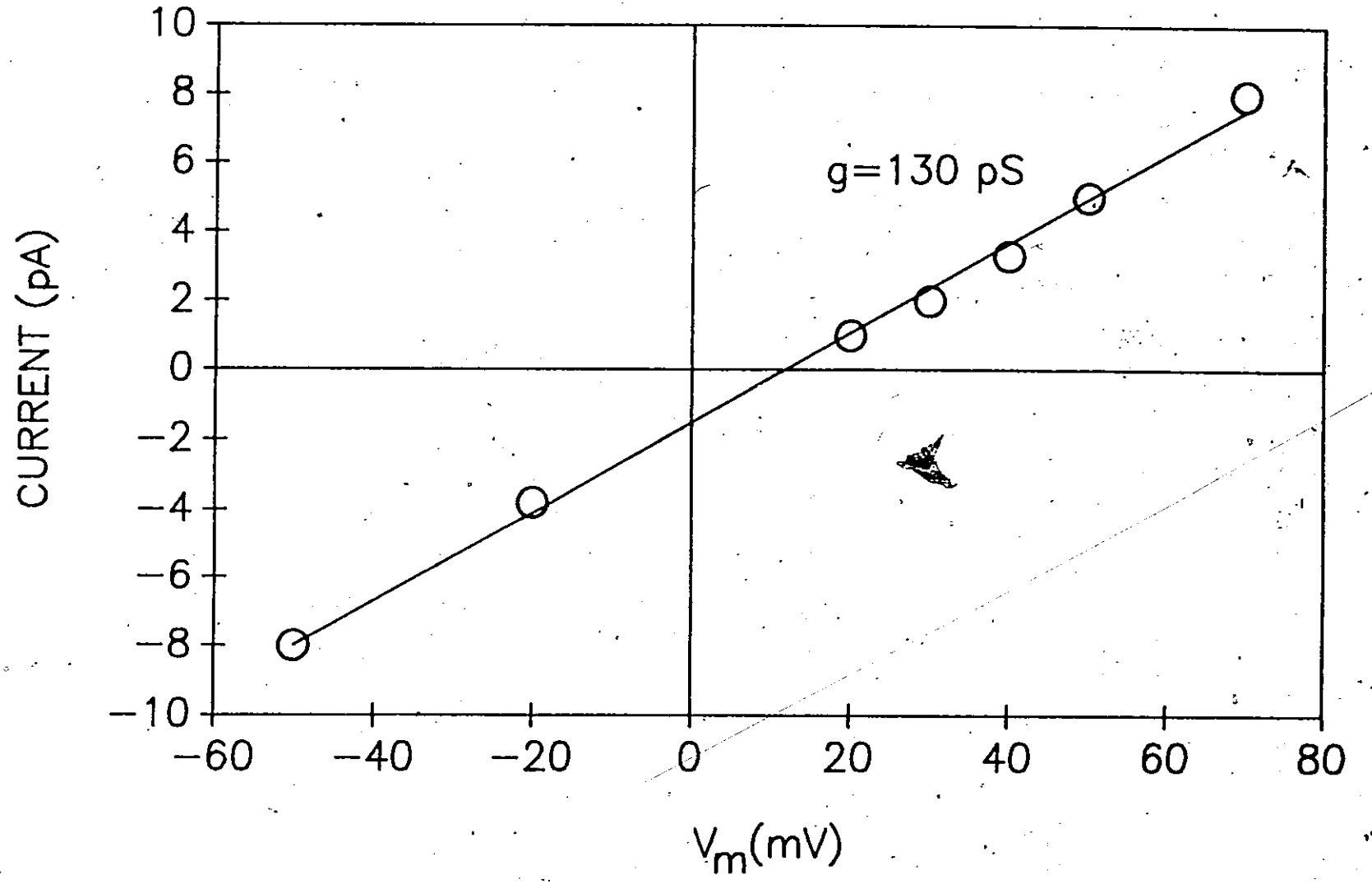
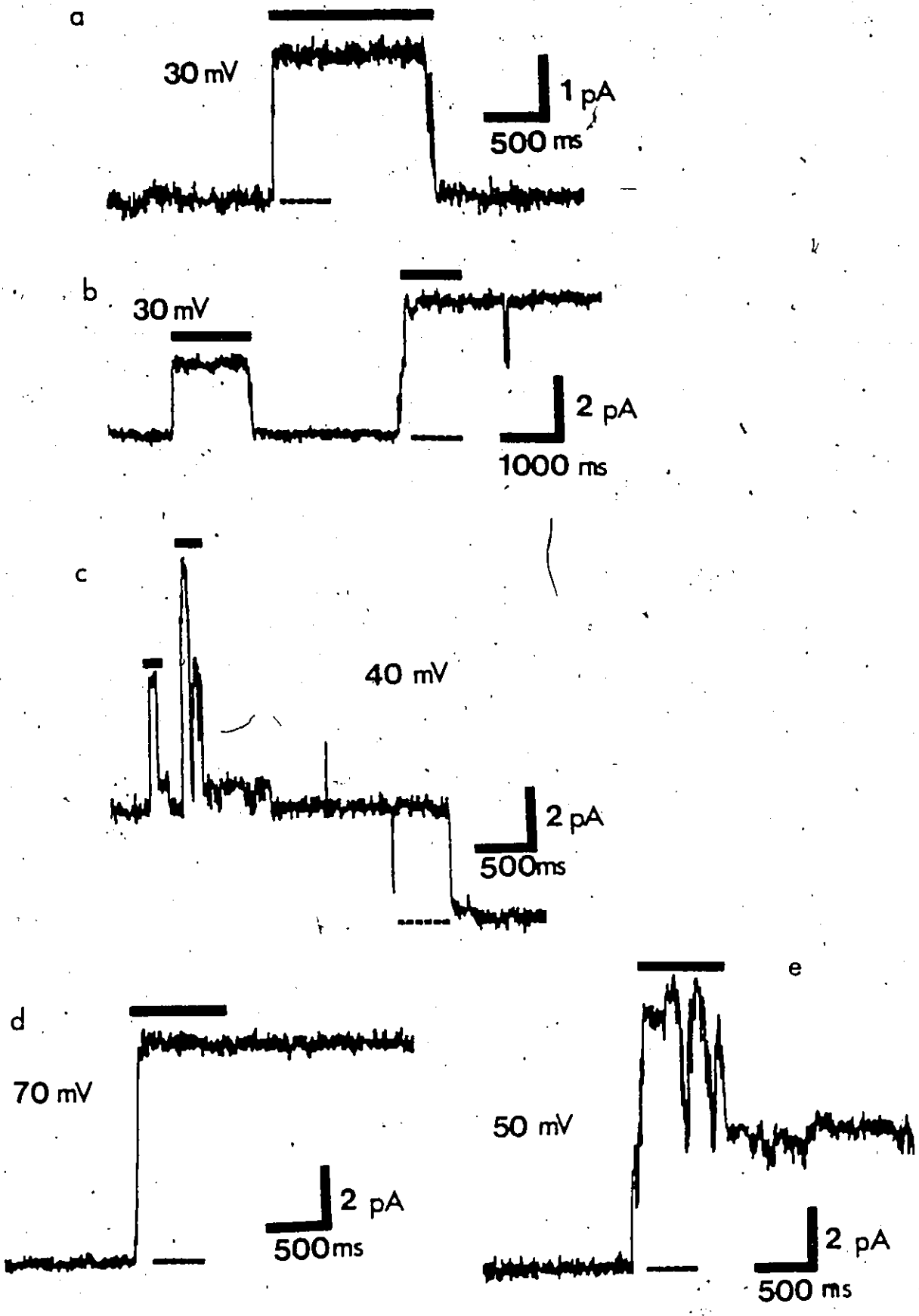


Fig.17 **Current-voltage relation for the modal state of the *Cepaea* SACl channel.** Inside-out patch; pipette solution, 70 mM K*; bath solution, NCS; Vr, 12 mV.


10
8
6
4
2
0
−2
−4
−6
−8
−10
CURRENT (pA)
g=130 pS
−60
−40
−20
0
20
40
60
80
V_m(mV)

cell-attached condition even when negative pressure was applied. Sensitivity to constituents of the solution on the cytoplasmic face (i.e. to Ca; Barish, 1983; Gelyetuk and Kazachenko, 1985; Findlay and Petersen, 1985) or sensitivity to the absence of the inhibitory molecule such as ATP (Noma, 1983; Ashcroft et al., 1987) may be what allows channel opening in excised condition. No spontaneous activity of the channel was, however, observed even in the excised condition without application of suction. In this sense it differs from the Cl channels mentioned earlier. The minimal negative pressure required to activate the channel was in the range of -35 to -40 mm Hg, regardless of voltages. Suction of this magnitude leads to channel activation in 83% of trials (total number of trials=22; N=1 patch). When suction was applied, the channel remained open as long as the pressure was sustained (fig.18). Suction could activate one or several conductance states. Upon suction release, channel closure was sometimes immediate (fig.18 a,b), but often not (fig.18 b,c,d,e). To characterize the timecourse of closure upon release of suction, the conditional probability of the channel of being open, P(O,t), at various times, t, following suction release was

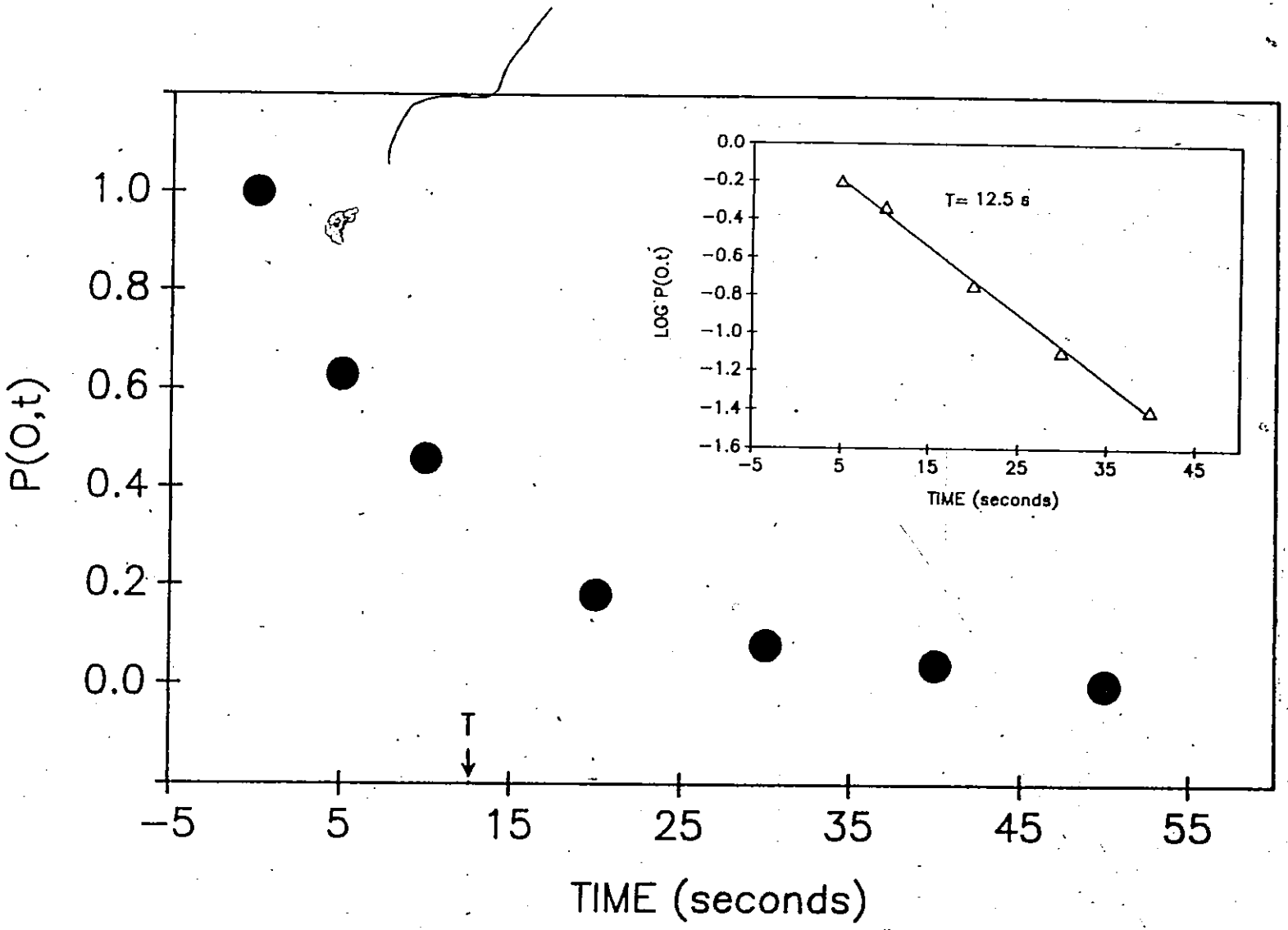
Fig. 18 **SACl channel behavior during transiently applied suction and following suction release.** Inside-out patches; pipette solution, 70 mM K*; bath solution, NCS; applied pressures, between -35 and -50 mm Hg; filter, 2 KHz. Dotted bars indicate the zero-current level. Solid bars indicate applied suction.


a
30 mV
1 pA
500 ms
b
30 mV
2 pA
1000 ms
c
40 mV
2 pA
500ms
d
70 mV
2 pA
500ms
e
50 mV
2 pA
500 ms

followed in one patch. P(O,t) declined with an exponential timecourse, whose decay constant was 12.5 s (fig. 19). It should be noted that the occasional brief closure (tens of milliseconds) was ignored in this analysis. The finding that a single exponential can describe the closure of the modal state of this channel indicates that, regardless of how complex the activation kinetics of the channel may be (see Hille, 1977), one process is rate-limiting for closing. Once the channel closed following suction release, a long closed period was not required to reactivate the channel with suction (fig. 18 c). Reactivation by suction could occur within 5 s following the channel closure.

Regardless of the voltage, suction generally activated the channel to the same conductance level which will be called the modal state. This modal state, which has a conductance of 130 pS (fig. 17) was not, however, the highest conductance observed in patches. Suction occasionally activated the channel to a level twice as great (260 pS; fig. 16 at -20 and 50 mV). The channel spends 93 $\pm$ 5% (S.D.) of its open time in the modal state (N=3 patches). When the channel was in a given conductance state, further

Fig. 19 Timecourse of the SACl channel closing after suction is released. P(O,t) is the conditional probability that given the channel is open at time t=0, it remains open at time t. The experimental points from 5 s after suction release can be represented by one exponential as shown by the linear curve of the log P(O,t)/time relation (inset; line is from a log-linear best-fit, regression coefficient, 0.98). The exponential time constant, T, derived from the fit is 12.5 s. The modal state was used to calculate P(O,t). Data were taken at various voltages. Note that the 0 time data is omitted from the fit (since it is fixed at a value of 1) and the last point is omitted (since its value, 0, has no log value).


P(O,t)
1.0
0.8
0.6
0.4
0.2
0.0
T
−5
5
15
25
35
45
55
TIME (seconds)
LOG P(O,t)
0.0
−0.2
−0.4
−0.6
−0.8
−1.0
−1.2
−1.4
−1.6
T= 12.5 s
−5
5
15
25
35
45
TIME (seconds)

application of suction could lead to the activation of one or several other states (fig.18 c). In such cases, the newly activated states usually became inactivated when suction was released, and, as illustrated in figure 18 (c), returned to the initial conductance level. During transitions between the major conductance states, the Cl channel displayed many small subdivisions of states (fig.20). The small, brief subdivisions were most frequently observed during transitions from one major conductance level to another. The greater noise level when the SACl channel is fully is open (130 pS; fig.20 a, c, d) also reflect these smaller subdivision states.

Some Cl channels have been reported to be voltage sensitive (Kolb, Brown and Murer, 1985; Welsh, 1986; Woll, Leibowitz, Neumcke and Hille, 1987; Woll and Neumcke, 1987). The opening and closing of the SACl channel is not, however, affected by depolarization or hyperpolarization. The probability that the channel opens in the first second after suction is applied, F(O), and the probability of closure within 1 second of suction release, F(C), change randomly with membrane potential (fig.21).

Fig.20 SAC1 channel small conductance states. Small conductance states are observed during transitions between the major (130 pS) conductance states (i.e. fully open or closed) (a, b, c), when the channel is already partially open (a,d), when the channel opens from the closed state (c; indicated by the arrow). The events are most often seen when suction is applied (a, b) but also, less frequently when suction is no longer being applied but the channel is still active (c, d). Inside-out patch; pipette solution, 70 mM K*; bath solution, NCS; applied pressure, -35 to -40 mm Hg; filter, 2 KHz; scale bar: a, b, c (1 pA, 20 s) and d (2 pA, 1s). Dotted bars indicate closed state. Solid bar indicate applied suction.

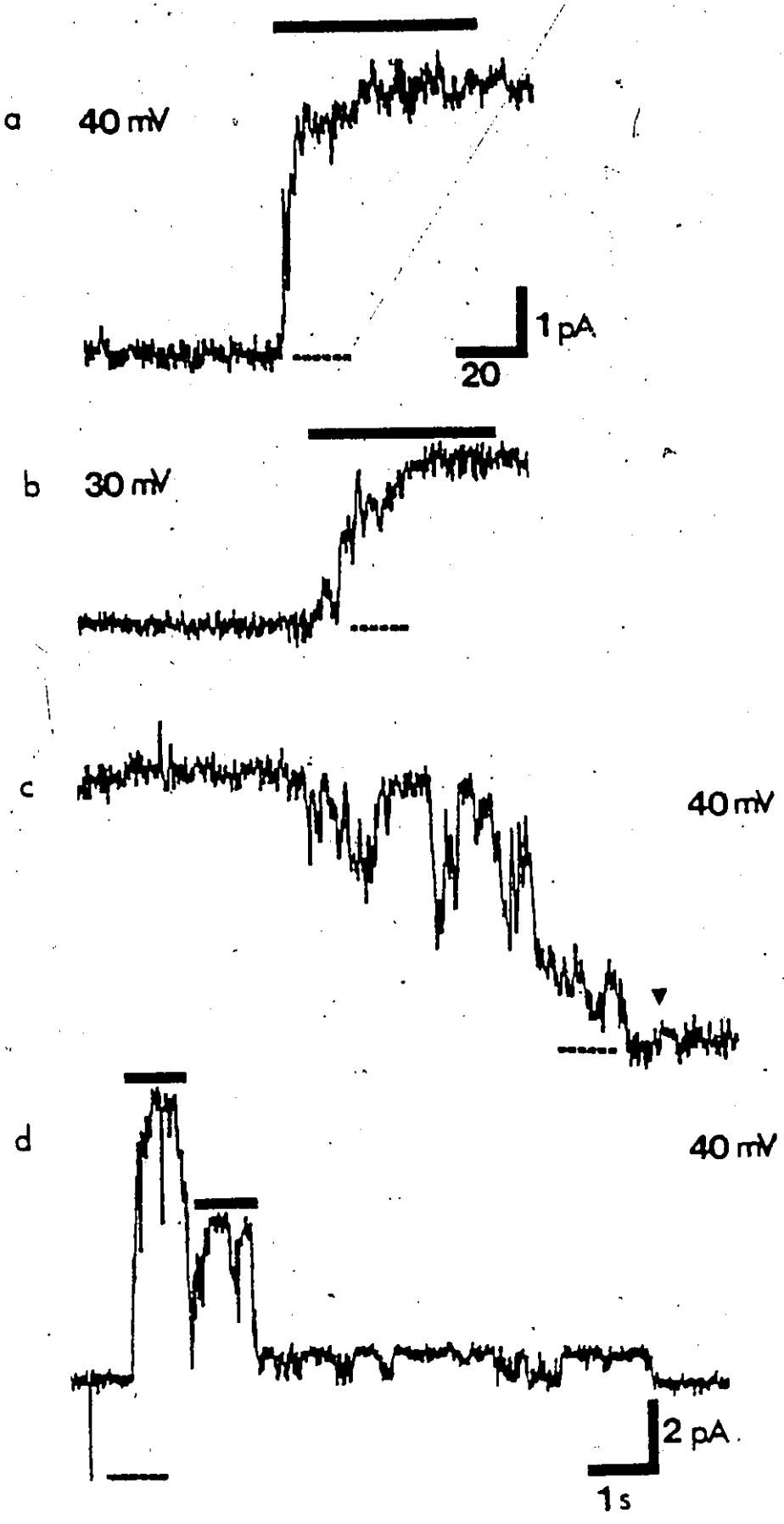
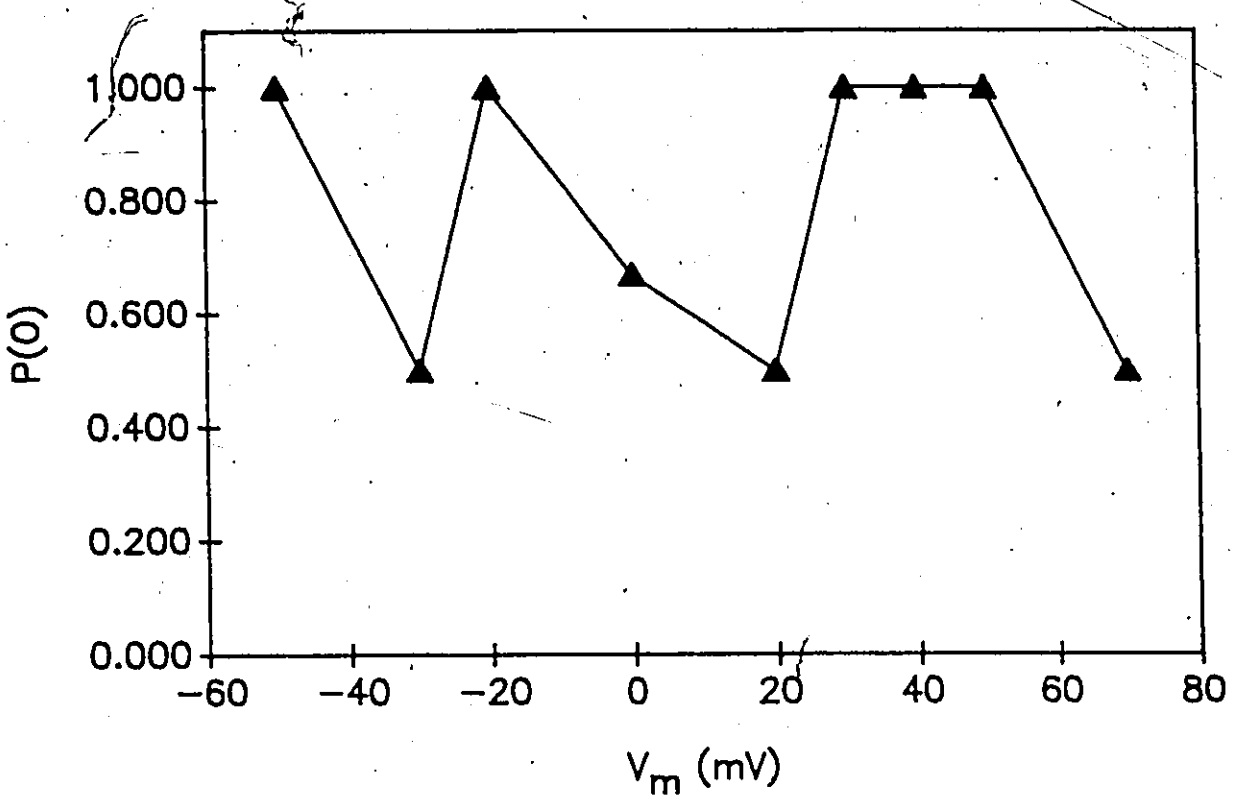
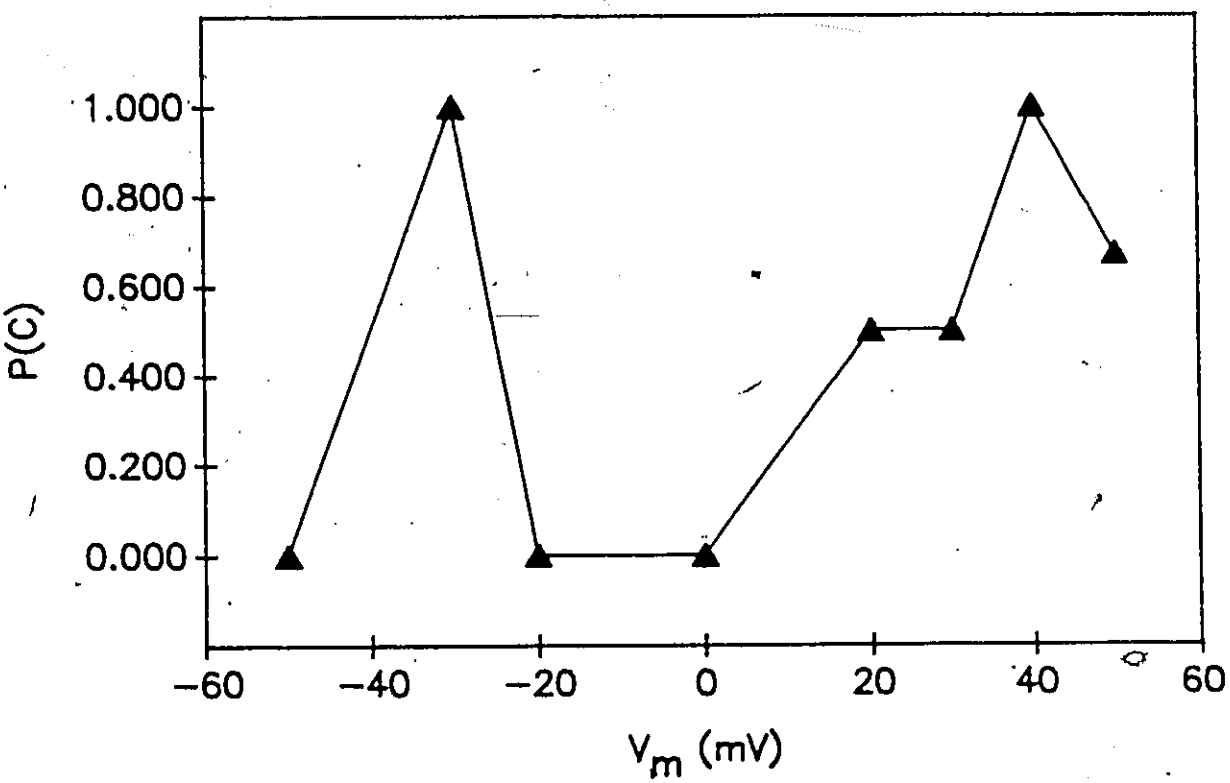

a
40 mV
1 pA
20
b
30 mV
c
40 mV
d
40 mV
2 pA
1 s

Fig.21 Probability of SACl channel opening or closing is not affected by voltage. Pressures between -35 to -40 mm Hg were applied 3-5 times at each voltage. A channel was counted as having opened or closed if an opening or closure occurred within 1 s following suction application or release, respectively. Inside-out patch; pipette solution, 70 mM K*; bath solution, NCS.


1.000
0.800
0.600
0.400
0.200
0.000
P(O)
−60
−40
−20
0
20
40
60
80
V_m (mV)


1.000
0.800
0.600
0.400
0.200
0.000
P(C)
−60
−40
−20
0
20
40
60
V_m (mV)

III.5 Leech and cockroach

Pressures between -100 and +50 mm Hg did not trigger the opening of a channel or change the activity of the observed channels in the cell body of leech and cockroach neurons. Applying positive pressures higher than 50 mm Hg was not possible because it would break the patch. Because some stretch-channels are mildly voltage-sensitive (Martinac et al, 1987; Methfessel et al., 1986), membrane potentials up to 120 mV were applied without observing any stretch-activated channels (Leech, N=22 patches; Cockroach, N=15 patches). Stretch was also applied to patches excised into solutions with calcium concentrations higher than 10^{-4}M on the inner face (i.e. normal saline) with high K in the pipette (Table 2). Failure to observe stretch-channel in cell-attached configuration cannot, therefore, be attributed to a requirement for calcium (as in the case of the Ca-activated K channel; Latorre and Miller, 1983; Yellen, 1984; Benham et al. 1986) which was unavailable in the cell. The absence of stretch-activated channels in the neuronal cell bodies of both animal species was not due to an inability to form adequate seals or to other trivial technical

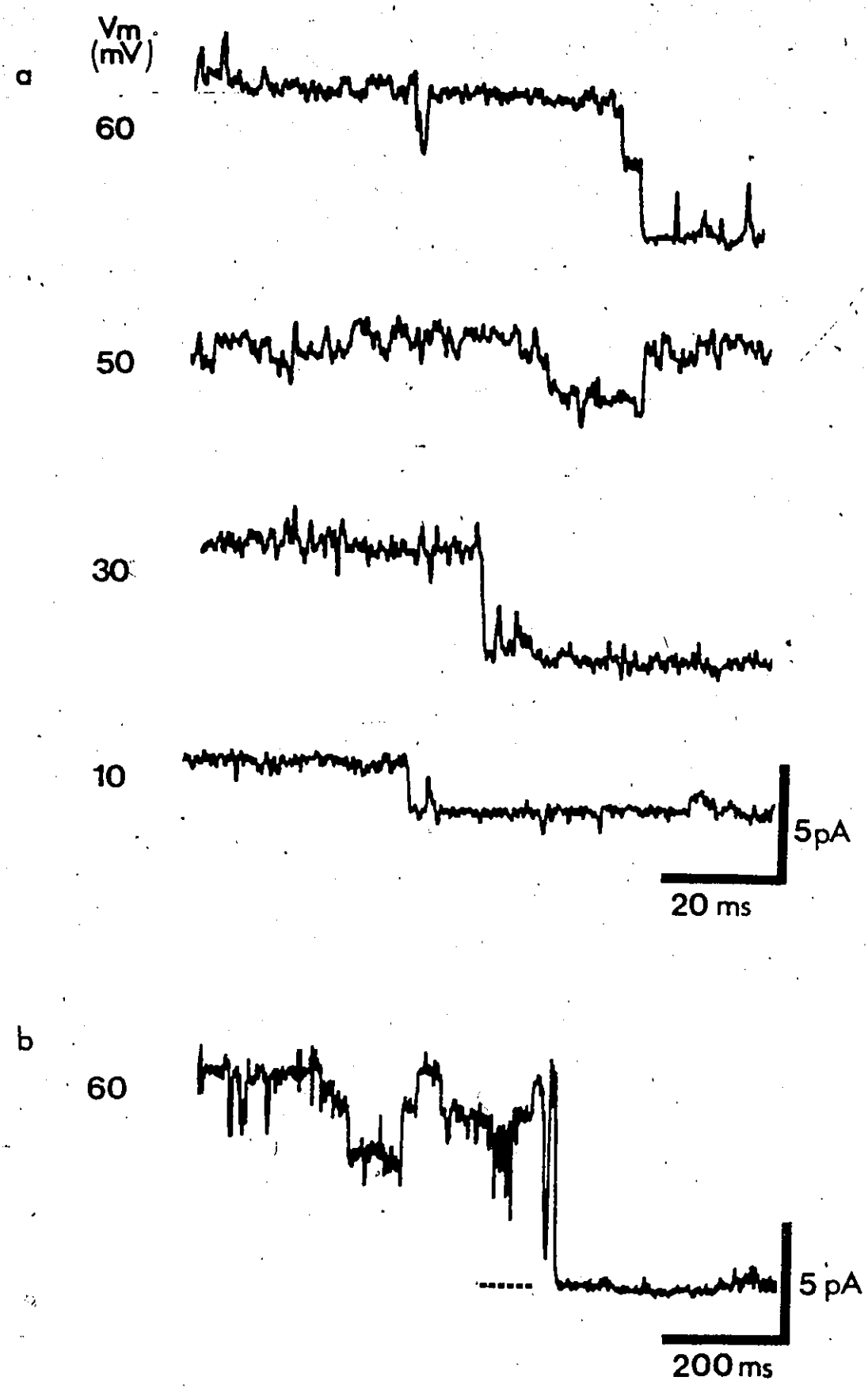
problems. Evidence for this is the presence of other channels in patches.

III.51 Cl channel in leech

In leech, a large conductance channel similar to those observed in pulmonary alveolar epithelial cell (Krouse et al, 1986), Lymnaea neuron (Geletyuk and Kazachenko, 1986) and Cepaea neuron was seen in 5 of 12 patches (fig. 22). In cell-attached condition with NHS in the pipette, the reversal potential (-50 mV) of the channel is near the resting potential, (Vr= -44 ± 5 mV; Deitmer and Schlue, 1981). In excised patches, low K concentration on both sides of the membrane (i.e. NHS; Deitmer et al., 1981) did not abolish the channel currents, suggesting a chloride channel. As expected for symmetrical conditions, the zero current potential is about 0 mV. Na is ruled out since the channel does not reverse at positive membrane potentials with NHS in the pipette in cell-attached conditions.

Channel activation occurred more frequently with membrane depolarization. Excision of the patch

Fig.22 Single channel currents of a large Cl channel in leech neuron. Inside-out patch; NHS on both side of the membrane; filter, 1 KHz. a) currents at various voltage. b) the Cl channel shows many conductance levels. Dashed line indicates zero current levels.


a
Vm
(mV)
60
50
30
10
5pA
20 ms
b
60
5 pA
200 ms

increased the probability of being opened. 3 patches out of 5 revealed the channel when inside-out patch experiments were performed. This effect could be due to the high Ca concentration in the bath (Geletyuk and Sachs, 1985; Findlay and Petersen, 1985) or to a decrease in the concentration of some inactivating molecule such as ATP (Noma, 1983; Ashcroft et al., 1987).

It is interesting to notice that this Cl channel was observed in the cell-attached condition (N=2 patches). Other similar channels have been seen only in the excised patch condition (Welsh, 1986; Krouse et al., 1986; Geletyuk and Kazachenko, 1985). Observation of this channel in the cell-attached condition indicates that the characteristics of the channel seen in tear off may be physiologically relevant, and not merely an artefact of exposure to a bizarre chemical environment.

III.52 Inward rectifier K channel in leech

Also in leech, a small flickery channel of 28 pS was observed. A similar channel has been seen in muscle cells (Biermans, Vereeke and Carmeli, 1987; Matsuda,

1988). The channel was observed in the cell-attached condition, with 40 mM K in the pipette (Table 2). The channel was only evident with hyperpolarization (fig.23). Whether this absence of currents with depolarization was due to the inactivation of the channel by depolarization (ie a gating phenomenon) or to an effect such as the blocking of the channel by Mg (Matsuda, 1988) was not determined. With NHS in the pipette, this channel was never observed. The extrapolated reversal potential of the channel with 40 mM K in the pipette and 101.4 mM K in the cell (Deitmer et al., 1981) was -13 mV (fig. 23 and 24), strongly suggesting that the channel is K selective since no other major ion in the system shares this reversal potential. The expected reversal potential for a K channel in these conditions, -23 mV, is relatively close to the estimated reversal potential. It is a property of inwardly rectifying K channels to activate only below their reversal potential (Matsuda, 1988).

The inward rectifier K channel observed in leech is not stretch sensitive (0 to -90 mm Hg). In endothelial cells, whole cell recording has shown an inwardly rectifying K (Kir) current which is activated by shear-

Fig.23 **Inwardly rectifying single-channel K currents observed in leech neuron.** Cell-attached; pipette solution, 40 mM K; filter, 2 KHz.

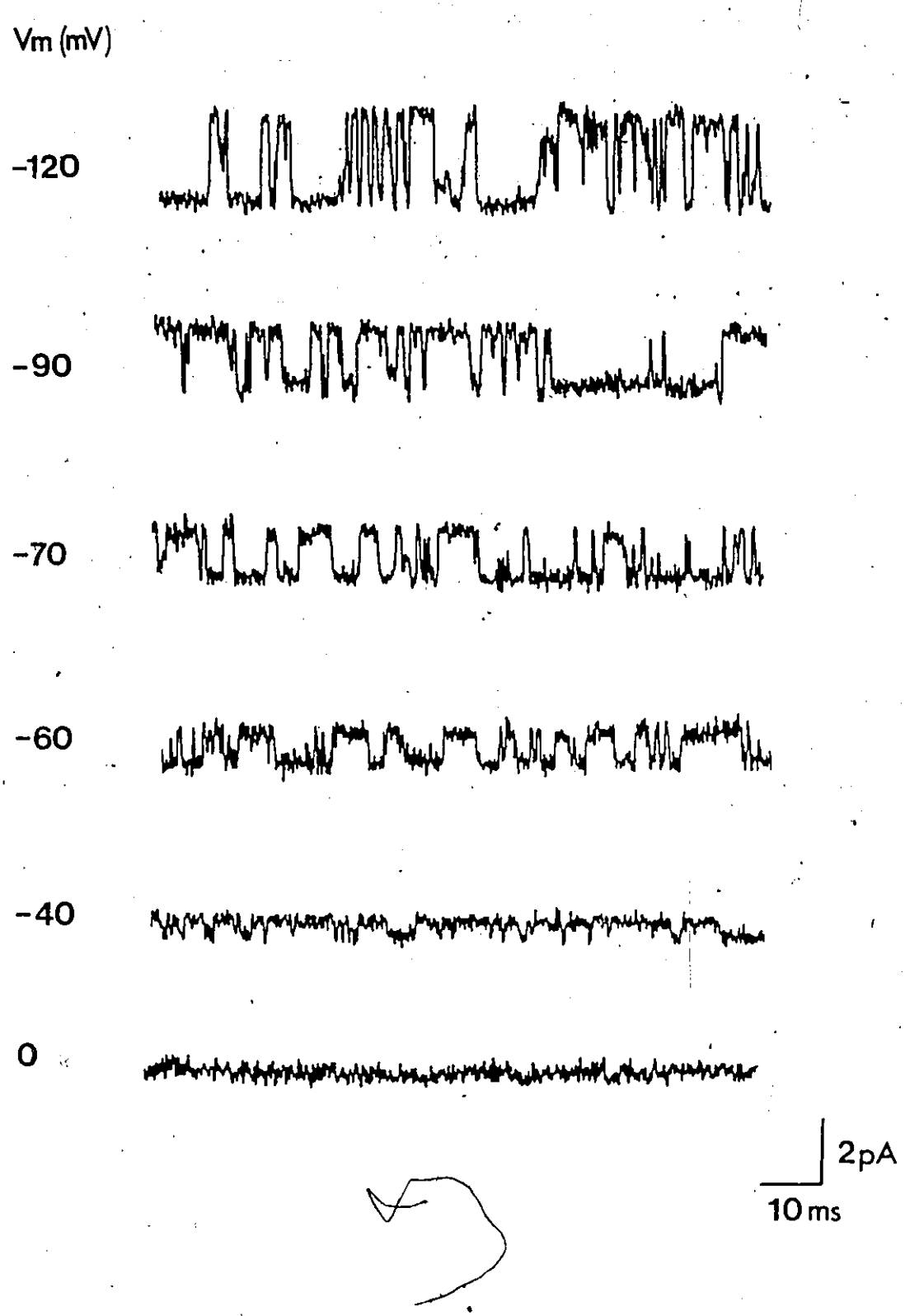
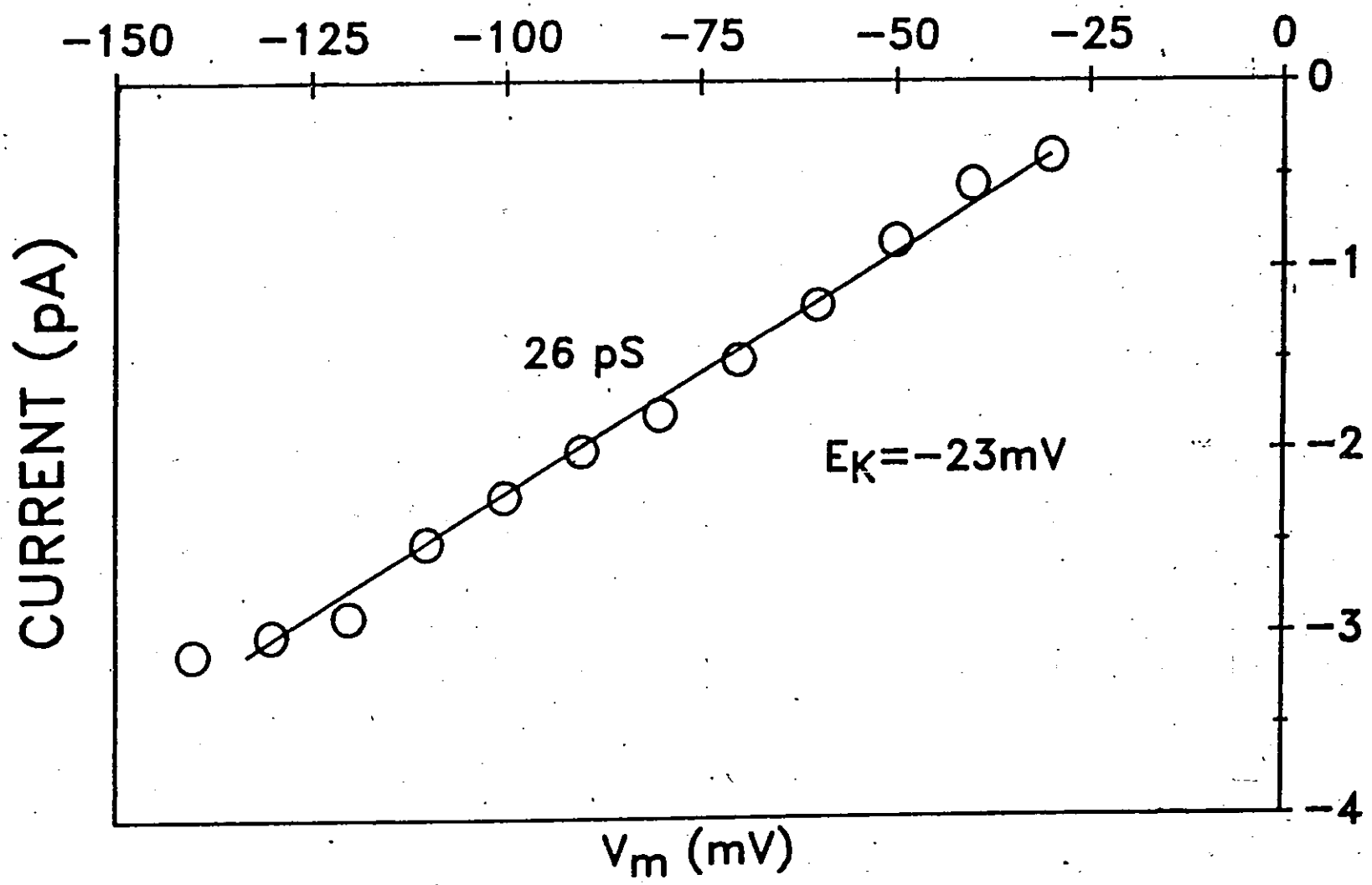

Vm (mV)
-120
-90
-70
-60
-40
0
2pA
10 ms

Fig.24 **Current-voltage relation for the K rectifier channel in leech.** Cell-attached; pipette solution, 40 mM K; extrapolated Vr, -13 mV; predicted Vr, - 23 mV (estimated using the values given in the text).


−150
−125
−100
−75
−50
−25
0
0
−1
−2
−3
−4
CURRENT (pA)
26 pS
E_K=−23mV
V_m (mV)

stress (Olesen et al., 1988). At the single-channel level, however, no Kir channels activated by stretch were observed. It is possible, then, that shear does not directly activate the endothelial Kir but instead activates some second messenger which then activates the Kir current.

III.53 K channels in cockroach

In cockroach neurons, 2 different ion channels were observed using NPS (Table 2) in the pipette in the cell-attached configuration. In this condition, two channels had their reversal potential near the resting potential (Vr=-50 mV) (Leech, 1986). With hyperpolarization, beyond rest, no currents were observed suggesting that both channels are K selective. (fig. 25 and 26). The channel conductances were 14 pS and 28 pS (N=2 patches). Larmet et al.(1988) has reported two K channels of similar conductance from Periplanata neurons. The two channels seen in the present study were not sensitive to the pressure in the range 0 to -80 mm Hg. The formation of poor gigaseals and the fragility of the cell membrane prevented the

Fig.25 Single-channel currents for the K channels seen in cockroach. Cell-attached; pipette solution, NPS; S, small K channel; L, large K channel; filtre, 2 KHz.

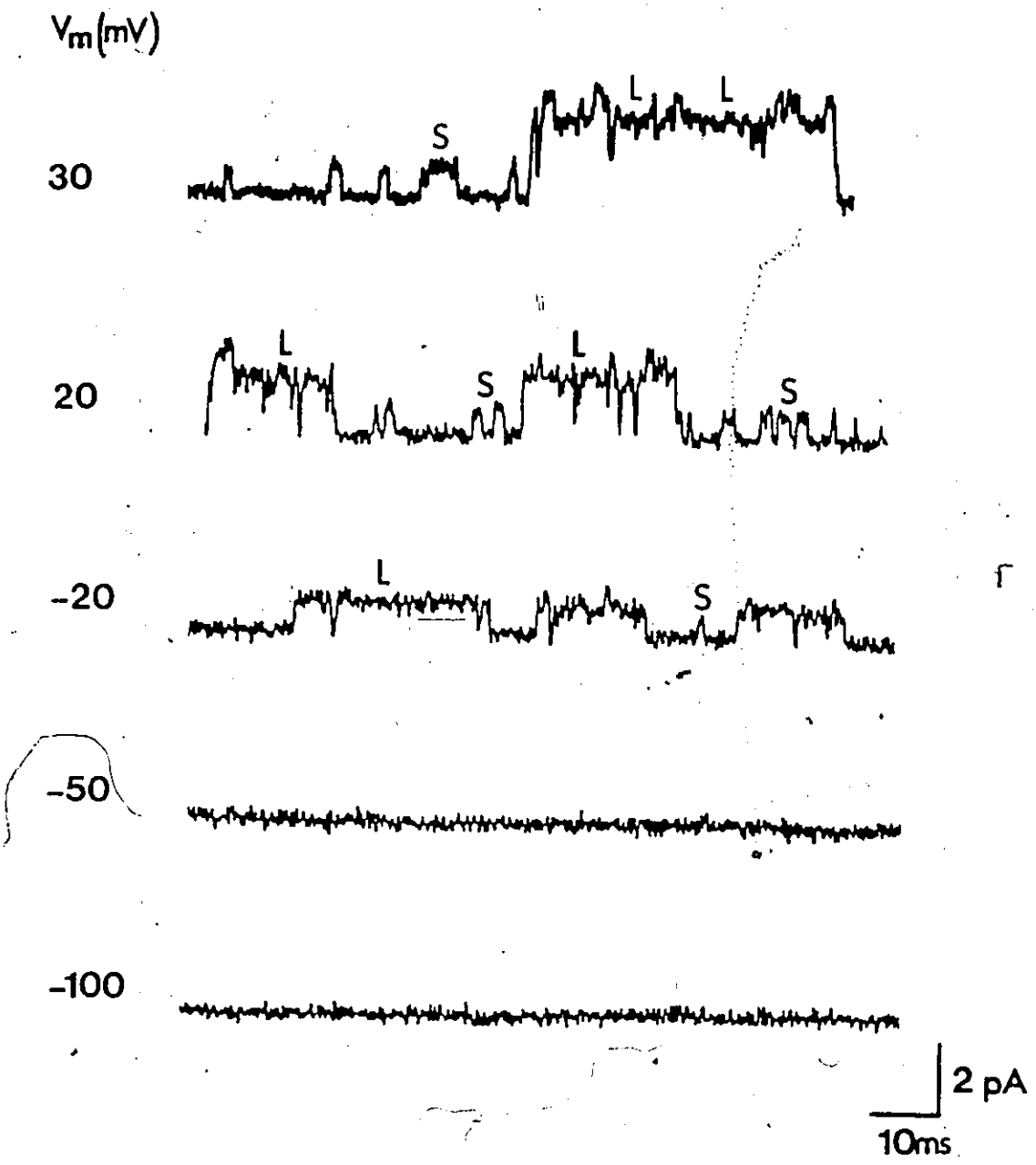
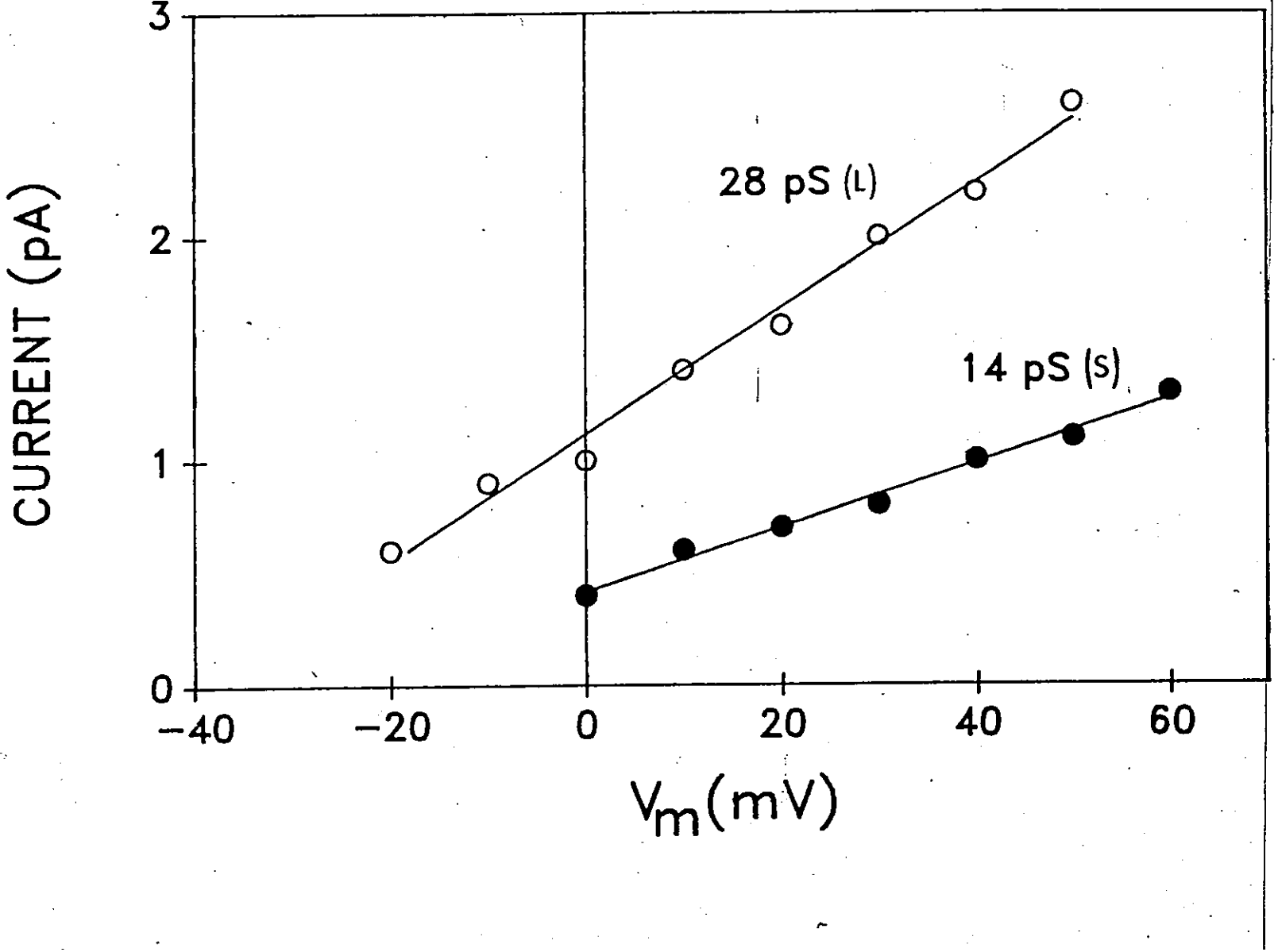

Vm(mV)
30
20
-20
-50
-100
S
L
L
L
S
L
S
L
S
2 pA
10ms

Fig.26 **Current-voltage relation for two K channels observed in cockroach neuron.** Cell-attached; pipette solution, NPS; Vr is between -50 and -40 mV.


CURRENT (pA)
3
2
1
0
28 pS (L)
14 pS (S)
−40
−20
0
20
40
60
V_m(mV)

application of higher negative pressures and positive pressures. Since this could explain the failure to observe stretch-activated channel, the possibility of SAK channels of low stretch-sensitivity (such as group II from Cepaea neurons) in cockroach neurons is not ruled out.

CHAPITRE IV

IV DISCUSSION

IV.1 La sensibilité à l'étirement des canaux SAK et SACl est-elle un phénomène réel ou artificiel?

La possibilité que la sensibilité à l'étirement des canaux SAK et SACl de Cepaea soit un phénomène artificiel est peu probable si on considère les observations suivantes: en absence de succion, l'activité des canaux est très basse pour le canal SAK et absente pour le canal SACl; parmi plusieurs types de canaux observés dans les patches, seuls les canaux SAK et SACl étaient affectés par la succion; l'ouverture des canaux suite à l'application de succion n'était pas occasionnelle mais se produisait dans 100% des essais pour le canal SAK et dans 83% des essais pour le canal SACl; les pressions appliquées sont physiologiques (30 mm de Hg=1.6 mosmoles). De plus, la présence du canal SAK dans 95% des patches, son insensibilité à d'autres modulateurs (le voltage est un modulateur mineur car l'activité du canal n'est affectée que vers +120 mV; voltage situé dans la région non-physiologique) et la découverte de canaux activés par l'étirement dans

divers types de cellules de différentes espèces animales suggèrent que la sensibilité à l'étirement des canaux SAK et SACl est un phénomène réel.

IV.2 Fonctions

IV.21 Régulation du volume cellulaire

- Canal SAK

Bien que les escargots terrestres vivent dans un environnement aride, ces derniers comme tout animal terrestre subissent des stress hypoosmotiques (ex.: pluie) qui nécessitent l'intervention d'un mécanisme de régulation du volume (Burton, 1983). L'une des stratégies d'osmorégulation utilisée par les escargots terrestres est de se réfugier dans leur coquille (Burton,1983). Chez l'invertébré marin Mytilus edulis la fermeture de la coquille est utilisée pour prévenir un stress hypoosmotique suite à un changement rapide de l'osmolarité (Treherne, 1980). Chez les escargots terrestres, la littérature ne mentionne pas si le retrait de l'animal dans sa coquille est utilisé comme

mécanisme de prévention à un éventuel choc hypoosmotique et/ou comme mécanisme de dernier recours pour arrêter une dilution plus ou moins avancée des fluides du corps. Advenant cette dernière éventualité, un mécanisme de la régulation du volume au niveau cellulaire est requis afin d'empêcher la lyse de la cellule. Dans les cellules de certains invertébrés (Treherne, 1980; Kevers, Pequeux and Gilles, 1981; Costa and Pierce, 1983; Moran and Pierce, 1984; Treherne, 1985) et vertébrés (Hoffman, 1985; Cala, 1983; Grinstein, Rothstein, Sarkadi and Gelfand, 1984; Ellory, Hall and Stewart, 1985), une perte de K accompagnée d'une sortie d'eau est observée lors d'un choc hypoosmotique. Chez Cepaea nemoralis, l'ouverture du canal SAK pourrait permettre une telle perte de K et permettre la régularisation du volume cellulaire. Dans les cellules des tubules rénales de Necturus, une augmentation de l'activité d'un canal sensible à l'étirement sélectif au K (Sackin, 1987) a été observée pendant le gonflement de la cellule sous stress hypoosmotique (Sackin, communication personnelle à C.E. Morris).

Canal SACl

Dans les cellules de certaines espèces animales, le Cl est le principal ion utilisé pour la régularisation du volume (Warren and Pierce, 1982; Grinstein et al, 1984). Chez Cepaea, le canal SACl tout comme le canal SAK pourrait aider à cette régularisation. Sous l'influence d'un choc hypoosmotique, une augmentation de l'activité d'un canal de Cl activé par l'étirement a été observée dans les cellules rénales d'opussum (Ubl et al., 1988).

L'activation du canal SACl chez Cepaea, en plus de l'étirement, semble nécessiter la présence d'un deuxième modulateur. Les divers changements intracellulaires se produisant dans la cellule sous l'influence d'un stress osmotique pourraient altérer la concentration de ce deuxième modulateur et permettre l'ouverture du canal. Grinstein et al (1984) ont suggéré que lors d'un choc hypoosmotique, une augmentation de la concentration de Ca provenant du bassin intracellulaire pourrait activer un courant de K dirigé vers l'extérieur (Grinstein et al, 1984). Dans les cellules du plexus choroïdien de la salamandre

Necturus maculosa, l'entrée de Ca via un canal cationique activé par l'étirement pourrait activer des canaux de K sensibles au Ca (Christensen, 1987).

Complémentarité des canaux SAK SACl

Dans les neurones et les fibres musculaires de crustacés (Freel, 1978; Treherne, 1980; Parker et Pierce, 1985), la perte de K causée par une diminution de la salinité entraîne une hyperpolarisation du potentiel de repos. Chez Cepaea nemoralis, une telle hyperpolarisation pourrait être évitée grâce à la présence des canaux SAK et SACl. La sortie de Cl et de K créant un flux électriquement neutre, permettrait le maintient du potentiel de repos.

IV.22 Autres fonctions

Le canal SAK de Cepaea pourrait jouer un rôle dans le processus de croissance cellulaire. La présence d'un canal SAK dans les cônes de croissance des neurones de Lymnaea stagnalis (Sigurdson et Morris, communication

personnelle) suggère que ce dernier pourrait aider à la régularisation de la croissance cellulaire. Chez les escargots, le processus d'allongement des cônes de croissance est dépendant du calcium et du voltage et pourrait ainsi nécessiter l'intervention de canaux de Ca dépendant du voltage (Kater, Mattson, Cohan and Connor, 1988). Advenant une croissance trop rapide des bras de croissance, le canal SAK pourrait être activé et entraîner une hyperpolarisation de la cellule, ce qui permettrait la désactivation des canaux de Ca dépendant du voltage. La diminution de la concentration interne de Ca ainsi obtenue permettrait le ralentissement de la croissance des bras.

IV.23 Absence de fonction

Avec le passage du milieu aquatique au milieu terrestre, l'escargot peut ne pas avoir perdu le canal SAK et ce, même si ce dernier n'est pas utilisé. Cependant une telle possibilité est peu probable si on considère la présence non pas d'un canal d'étirement mais de deux canaux d'étirement dans les neurones de Cepaea, la présence d'un canal SAK chez l'amphibien

Necturus (Sackin, 1987), dans les astrocytes de mammifères (communication personnelle de Sachs à Morris) et dans les cellules musculaires de la Drosophile melanogaster(Zagotta et al., 1988).

IV.3 Similarités entre le canal SAK de *Cepaea nemoralis* et de *Lymnaea stagnalis*.

Le canal SAK de Cepaea a des propriétés communes avec le canal SAK retrouvé chez Lymnaea* (il est à noter que le canal SAK de l'amphibien Necturus# sera peu considéré dans cette comparaison dû au peu de données concernant sa caractérisation; lorsqu'une propriété touchera Necturus, un (N) sera indiqué). Il est fortement sélectif au K (N) et possède une constante de perméabilité élevée. Il est sensible à la quinidine et insensible au Ca, Na, TEA externe et 4-AP externe. Le canal est "flickery" (N). Il possède une longue constante de temps pour l'état fermé qui est sensible à

*Références pour Lymnaea stagnalis: Brezden et al, 1985; Sigurdson et al., 1986.

#Référence pour Necturus: Sackin, 1987.

l'étirement. Il est aussi légèrement sensible au voltage. Ces caractéristiques communes aux deux canaux SAK suggèrent qu'ils possèdent des structures similaires et appartiennent à la même famille de canal activé par l'étirement.

IV.4 Sangsue et blatte

Le fait de n'avoir détecté aucun SAK dans les corps cellulaires des neurones de la sangsue et de la blatte ne signifie pas que ces derniers ne sont pas présents chez ces animaux. Il est possible que les différentes techniques utilisées dans la présente recherche n'est pas permis d'identifier des canaux SAK qui pourraient avoir des caractéristiques particulières, caractéristiques non-observées dans les canaux SAK connus jusqu'à présent. De plus, le fait de ne pas avoir détecté le canal SAK dans les corps cellulaires des neurones de la sangsue et de la blatte n'exclue pas leur présence dans d'autres types de cellules ou dans d'autres parties du neurone telles l'axone et les cônes de croissance. Chez Lymnaea stagnalis, un canal SAK, similaire à celui retrouvé dans les corps cellulaires

de ces mêmes cellules, a été observé dans les cônes de croissance (Morris,C.E. et Sigurdson, W. J., communication presonnelle). Il est à noter que les axones ou cônes de croissance des neurones de la sangsue et de la blatte n'ont pu être patchés dans les présentes expériences en raison de leurs faibles proliférations ou de leurs faibles épaisseurs.

Le jeune âge des cellules nerveuses patchées (1 journée après isolation) pourrait expliquer l'absence de canaux SAK chez la sangsue et la blatte. Cependant, une telle possibilité est peu probable si on considère que dans les neurones de Lymnaea stagnalis et de Cepaea nemoralis , le canal SAK a été observé, respectivement, 2 heures (Morris et Sigurdson, communication personnelle) et moins de 24 heures après l'isolation.

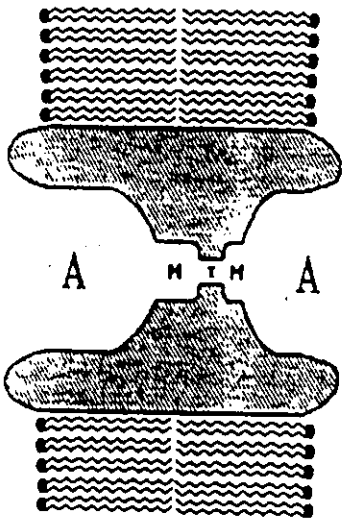
IV.5 Conformation du canal SAK

Parmi la grande variété de canaux de K (30 types qui diffèrent selon leur mode de régulation; Yellen, 1987), Latorre et Miller ont distingué deux catégories de canaux: les canaux maxi de K (grande conductance) et les petits canaux de K (Latorre et Miller, 1983).

Tout comme les grands canaux de K activés par le calcium, Ca(K), (Barret, Magleby and Pallotta, 1982; Latorre, Vergara and Hidalgo, 1982; Wong, Lecar and Alder, 1982; Benham et al., 1986; Lang and Ritchie, 1987) et le canal de K du réticulum sarcoplasmique, SR (Coronado et al., 1982; Coronado, Rosenberg and Miller, 1980), le canal SAK est un canal à grande conductance et appartient aux canaux maxi de K. La valeur de l'indicateur arbitraire de la grandeur du SAK défini comme le rapport de la conductance du canal sur la concentration symétrique de K utilisée pour calculer cette conductance; g/[K]sym), est de 1.4 (g=90 pS; [K] sym=70 mM) et se situe dans la marge des valeurs g/[K] sym des canaux maxi de K (g/[K] sym >1). Une valeur située sous 0.1 aurait dû être obtenue pour un petit canal de K (voir Latorre et al (1983) pour les valeurs de g de [K] sym).

La structure du canal SAK pourrait être similaire à celle proposée originallement par Miller et Latorre pour les grands canaux Ca(K) et SR (fig.27) (Yellen, 1987). Afin de réconcilier la haute sélectivité des grands canaux avec le haut taux de transport des ions, le modèle suggère un filtre de sélectivité court et

Fig. 27 Model of Maxi K channel. The tunnel (T), mouth (M), and antechamber (A) are parts of the hypothetical structure for K channel (from Yellen, 1987).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étroit bordé de chaque coté par de grandes antichambres. L'imperméabilité du canal SAK au Na, au TEA et au 4-AP, sa haute sélectivité au K et sa haute valeur de Pk supportent l'idée d'un filtre étroit et court. La présence d'antichambres permettant la formation de bassin de K de chaque coté du filtre est supportée par la haute valeur du Pk. En comparaison avec le grand canal SR (Coronado et al., 1980), la conductance du canal SR sature à une concentration relativement plus faible ($g_{1/2}$ SR=3 M; $g_{1/2}$ SAK=40 mM), ce qui suggère une structure des antichambres du canal SAK différente de celle du canal SR. Une telle différence pourrait être attribuée à un plus grand nombre de charges négatives dans l'antichambre et/ou à une plus petite grandeur de l'antichambre du canal SAK.

IV.6 Canal SAK et autres canaux de K

La différence majeure entre le canal SAK et le grand canal Ca(K) se situe au niveau de leur sensibilité au calcium et au voltage. L'ouverture du canal SAK n'est pas affecté par des concentrations de calcium (10^{-3} M) qui permettent l'activation des canaux Ca(K) (Barret et al., 1982; Benham et al., 1986; Lang and Ritchie,

1987). De plus, le canal SAK est insensible au potentiel membranaire. Seules de fortes dépolarisations situées hors de la région physiologique permettent une légère augmentation de l'activité du canal SAK.

Le canal SAK possède des caractéristiques pharmacologiques similaires à celles du canal de K sensible à la sérotonine (canal S) retrouvés dans les neurones sensorielles de l'escargot marin Aplysia california (Shuster and Siegelbaum, 1987). L'application externe de 10^{-3} M TEA et de 10^{-3} M 4-AP, concentrations qui inhibent les courants des canaux Ca(K), n'ont pas d'effets sur les courants unitaires du canal SAK. Le canal S est aussi tout comme le canal SAK un canal à grande conductance (Shuster and Siegelbaum, 1987).

Les éléments structurals des canaux SAK, S et Ca(K) permettant la haute sélectivité au K et leur grande conductance peuvent indiquer que ces trois canaux ont évolué d'un même canal de K. Chez la Drosophile melanogaster, il a été démontré que plusieurs types de canaux de K proviennent d'un même gène (Miller, 1988).

Ces canaux dont la région centrale est identique (région correspondant au filtre de canal) possèdent des régions distales construites d'oligomères de sous-unités différentes. Ces régions distinctes pourraient conférer à chaque classe de canaux de K sa modulation spécifique (Miller, 1988).

ANNEXES

Table 1

Cepaea nemoralis

Chemicals (mM)	NCS	70 mM K solution	40 Mm K solution	150 mM K solution	Choline, Cl solution	Low Ca solution	70 mM K* solution
NaCl	80	14	44	----	----	80	----
KCl	4	70	40	150	4	4	70
$CaCl_2 \cdot 2H_2O$	10	10	10	10	10	0.2	----
$MgCl_2 \cdot 6H_2O$	5	5	5	5	5	5	----
choline Cl	----	----	----	----	80	----	----
EGTA	----	----	----	----	----	2.2	----
HEPES	5	5	5	5	5	5	5
glucose	10	----	----	----	----	----	----

pH 7.8; glucose was not added to the NCS used for single channel recording. It was used only for cell culture.

Table 2

Leech and cockroach

Chemicals (mM)	NHS (Hirudo)	KCS (Hirudo)	NPS (Periplanata)
NaCl	115	75	200
KCl	4	40	3
$CaCl_2 \cdot 2H_2O$	1.8	1.8	4
$MgCl_2 \cdot 6H_2O$	----	----	5
Tris maleate	10	10	----
HEPES	----	----	10
Glucose	----	----	15
pH	7.4	7.4	7.2

Glucose was not added to the normal saline used for single channel recording. It was used only for cell culture.

Abbréviations

1.1 Composés chimiques et solutions salines.

Abbréviation	Signification
TEA	Tétraéthylamonium
4-AP	4-aminopyridine
NCS	Normal *Cepaea* saline
NHS	Normal *Hirudo* saline
NPS	Normal *Periplanata* saline

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