Arachidonic acid activation of a new family of K^+ channels in cultured rat neuronal cells

Donghee Kim, Celia D. Sladek, Carmen Aguado-Velasco and Joanne R. Mathiasen

Department of Physiology and Biophysics, Chicago Medical School, 3333 Green Bay Road, North Chicago, IL 60064, USA

1. The presence and properties of K^+ channels activated by arachidonic acid were studied in neuronal cells cultured from the mesencephalic and hypothalamic areas of rat brain.

2. Arachidonic acid produced a concentration-dependent (5–50 μM) and reversible activation of whole-cell currents.

3. In excised membrane patches, arachidonic acid applied to the cytoplasmic or extracellular side of the membrane caused opening of three types of channels whose current–voltage relationships were slightly outwardly rectifying, inwardly rectifying and linear, and whose single channel slope conductances at +60 mV were 143, 45 and 52 pS, respectively.

4. All three currents were K^+ selective and blocked by 2 mM Ba^{2+} but not by other K^+ channel blockers such as tetraethylammonium chloride, 4-aminopyridine and quinidine. The outwardly and inwardly rectifying currents were slightly voltage dependent with higher channel activity at more depolarized potentials.

5. Arachidonic acid activated the K^+ channels in cells treated with cyclo-oxygenase and lipoxygenase inhibitors (indomethacin and nordihydroguaiaretic acid), indicating that arachidonic acid itself can directly activate the channels. Alcohol and methyl ester derivatives of arachidonic acid failed to activate the K^+ channels, indicating that the charged carboxyl group is important for activation.

6. Certain unsaturated fatty acids (linoleic, linolenic and docosahexaenoic acids), but not saturated fatty acids (myristic, palmitic, stearic acids), also reversibly activated all three types of K^+ channel.

7. All three K^+ channels were activated by pressure applied to the membrane (i.e. channels were stretch sensitive) with a half-maximal pressure of ~18 mmHg. The K^+ channels were not blocked by 100 μM $GdCl_3$.

8. A decrease in intracellular pH (over the range 5·6–7·2) caused a reversible, pH-dependent increase in channel activity whether the channel was initially activated by arachidonic acid or stretch.

9. Glutamate, a neurotransmitter reported to generate arachidonic acid in striatal neurons, did not cause activation of the K^+ channels when applied extracellularly in cell-attached patches.

10. It is suggested that the K^+ channels described here belong to a distinct family of ion channels that are activated by either fatty acids or membrane stretch. Although the physiological roles of these K^+ channels are not yet known, they may be involved in cellular processes such as cell volume regulation and ischaemia-induced elevation of K^+ loss.

Ionic currents have been reported to be enhanced or depressed by arachidonic acid or its metabolites in various cell types (Meves, 1994). For example, the FMRFamide-activated K^+ channel (S-channel) activity in *Aplysia* sensory neurons is increased by arachidonic acid or one of its metabolites derived via the lipoxygenase pathway (Piomelli *et al.* 1987). In mouse neuroblastoma cells, *cis*-fatty acids were reported to depress both Na^+ and Ca^{2+} currents via protein kinase C (Linden & Routtenberg, 1989). In hippocampal CA1 pyramidal cells, arachidonic

acid was shown to depress Ca^{2+} current via both protein kinase C and generation of free radicals (Keyser & Alger, 1990). Arachidonic acid or its metabolites were reported to mediate somatostatin-induced enhancement of M-current in pyramidal neurons (Schweitzer, Madamba & Siggins, 1990), to mediate γ-aminobutyric acid-induced opening of K^+ channels in hippocampal cells (Premkumar, Gage & Chung, 1990), to inhibit Na^+ current and thereby depress synaptic transmission in cultured striatal neurons (Fraser, Hoehn, Weiss & MacVicar, 1993) and to enhance *N*-methyl-D-aspartate-activated currents (Miller, Sarantis, Traynelis & Attwell, 1992). Arachidonic acid that is generated by stimulation of dopamine receptors in the striatum has been suggested to be involved in synaptic depression as well as D1–D2 synergism (Piomelli, Pilon, Giros, Sokoloff, Martres & Schwartz, 1991; Calabresi, Maj, Pisani, Mercuri & Bernardi, 1992). Arachidonic acid has also been suggested to act as a possible retrograde messenger that is responsible for augmented presynaptic transmitter release (Kandel & O'Dell, 1992).

Recent studies in cardiac and smooth muscle cells have shown the existence of ion channels which are normally closed but are activated by free fatty acids. So far, the channels activated by a free fatty acid have been shown to be K^+ selective. In rat cardiac myocytes and in toad gastric smooth muscle cells, application of a free fatty acid such as arachidonic acid to the membrane activated K^+-selective ion channels with properties distinct from other previously identified K^+ channels (Kim & Clapham, 1989; Ordway, Walsh & Singer, 1989). Since the K^+ channels can be activated in excised inside-out patches in the absence of cytosolic constituents, the fatty acids presumably act directly on the channel or a regulatory associated protein. At present, how a fatty acid activates an ion channel remains unknown. Interestingly, the K^+ channel activity was enhanced by a decrease in intracellular pH as well as by stretch (Wallert, Ackerman, Kim & Clapham, 1991; Kim, 1992). Membrane stretch not only increased the frequency of channel opening of an already active K^+ channel but also opened inactive K^+ channels. Membrane stretch and fatty acids were also found to activate Ca^{2+}-activated K^+ channels in vascular smooth muscle cells (Kirber, Ordway, Clapp, Walsh & Singer, 1992) and the S-channel in *Aplysia* sensory neuron (Vandorpe, Small, Dabrowski & Morris, 1994). Thus, there may exist a common class of ion channels that are sensitive to both fatty acids and stretch.

The goal of the present study was to investigate whether neuronal cells from rat brain also possess K^+ channels that are activated by free fatty acids and sensitive to membrane stretch and changes in pH. If such K^+ channels are present, they may play an important role in neuronal function. For these studies, we used cultured cells prepared from the mesencephalic and hypothalamic areas of rat brain. The results of our study show that the neuronal cell membrane possesses three types of K^+ channel that belong to a new class of fatty acid-activated K^+ channels.

METHODS

Cell preparation

Hypothalamic and mesencephalic tissues were microdissected from 14-day-old fetal Sprague–Dawley rats using methods similar to that described earlier (Collier, Sladek, Gallagher, Gereau & Springer, 1990; Sladek & Gallagher, 1993). Adult female rats were anaesthetized with 2% halothane, and fetuses were rapidly decapitated. The tissue was collected in Ca^{2+}- and Mg^{2+}-free buffer solution and incubated in 0·1% trypsin solution at 37 °C for 10 min. Dispersed cells after gentle agitation were collected and plated at 10^5 cells per 16 mm well in Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum, 0·6% glucose, 0·1% L-glutamine, 100 u ml^{-1} penicillin, 100 mg ml^{-1} streptomycin and 2·5 mg ml^{-1} fungizone. The cells were placed in an incubator gassed with 5% CO_2–95% air at 37 °C. Culture medium was changed every 2 days. Electrophysiological experiments were done on cells grown for 2–5 days.

Electrophysiology

Cell bodies of neurons in culture were identified by phase-bright appearance of cells with two or more processes (see Fig. 1) and gigaseals were formed with Sylgard (Dow Corning)-coated thin-walled borosilicate pipettes with ~3 MΩ resistances. Channel currents were recorded using the method described by Hamill, Marty, Neher, Sakmann & Sigworth (1981). Channel current recorded with an Axopatch 1D patch-clamp amplifier (Axon Instruments, Foster City, CA, USA) was filtered at 10 kHz using an eight-pole Bessel filter (902-LPF; Frequency Devices, Inc., Haverhill, MA, USA), digitized by a PCM adapter (VR10; Instrutech Corp., Great Neck, NY, USA; frequency response, 37 kHz), and stored on videotape using a videotape recorder (JVC). The recorded signal was transferred directly in digital form to an Atari ST computer using the VCATCH program (Instrutech Corp.), and continuous single channel currents were analysed with the TAC program (Instrutech Corp.). The built-in Gaussian digital filter was set at 2 kHz (equivalent to a Bessel filter with −3 dB bandwidth) for analysis of open time duration. At this setting of 2 kHz, the minimum detectable event duration is ~80 ms (1/6 of a cycle of filter frequency) when the 50% threshold detector is used. After obtaining all open time events, durations less than 100 ms were deleted for plotting the open time histogram. The A/D board (ITC16-ST computer) used was a 16-bit A/D converter which has linearity up to 100 kHz. Data were analysed to obtain duration and amplitude histograms and channel activity (averaged NP_o) using the analysis protocol described by Sigworth & Sine (1987). N is the number of channels, and P_o is the probability of channel opening. Logarithmic (abscissa) and square root (ordinate) scales were used to represent dwell time distributions. Single channel dwell times were plotted on a logarithmic time scale using binned maximum likelihood fitting with constant logarithmic bin width (10 bins $decade^{-1}$). Burst duration was determined by setting the gap duration at 5 ms. All experiments were performed at 24–26 °C. All values are represented as means ± S.D.

Solutions and materials

For whole-cell experiments, the bath solution contained (mM): 135 NaCl, 10 KCl, 2 $MgSO_4$, and 10 Hepes (pH 7·2). In

experiments using excised patches, the standard bath and pipette solutions contained (mM): 140 KCl, 1 EGTA, 2 $MgCl_2$, and 10 Hepes (pH 7·2). In some experiments, bath K^+ was replaced with an equimolar concentration of Na^+, Li^+, Cs^+ or choline to determine cation selectivity. To determine anion selectivity, Cl^- was replaced with glutamate. To change solutions, the pipette tip with the attached membrane patch was brought to the mouth of a polypropylene tubing through which flowed the desired solution at a rate of ~1·0 ml min^{-1}.

ATP, glibenclamide, apamin, amiloride, tetraethylammonium, 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid (DIDS), quinidine and tetrodotoxin were purchased from Sigma Chemical Co. (St Louis, MO, USA). Nifedipine was purchased from Calbiochem (La Jolla, CA, USA). Gadolinium chloride ($GdCl_3$) was purchased from Aldrich (Milwaukee, WI, USA). All free fatty acids purchased from Sigma were kept at −70 °C until used. Immediately before each experiment, the organic solvent in vials containing the fatty acid was evaporated under pure N_2 at 0 °C, and bath solution was added to the vials to the desired final concentrations. The solution was sonicated for 15 s and used immediately. Pressure in the pipette was changed as follows: one end of a mercury manometer was connected to the pipette holder with a plastic tubing and the other end was open to air. Changes in pipette pressure could be achieved rapidly and accurately to the desired levels by operating the syringe connected to the tubing between the pipette and manometer.

RESULTS

Activation of whole-cell currents by arachidonic acid

Mesencephalic and hypothalamic neurons grown in culture on glass coverslips are shown in Fig. 1. Cell bodies of neurons were easily identified by their phase-bright round and oval shapes. We first studied whether arachidonic acid could activate an ionic current under the whole-cell condition. Whole-cell membrane potential was held at −60 mV relative to the reversal potential (cell membrane potential, approximately −120 mV) to record inward current. Arachidonic acid applied extracellularly to a mesencephalic neuron elicited a slowly developing inward current that was blocked by 2 mM Ba^{2+}, a non-specific K^+ channel blocker (Fig. 2). The increase in inward current produced by 50 μM arachidonic acid was 0·54 ± 0·12 nA ($n = 6$). Arachidonic acid also increased the outward current when the cell membrane potential was held at −40 mV. The reversal potential was not shifted and remained at approximately −63 ± 3 mV ($n = 4$). Similar activation of inward and outward currents was present in hypothalamic neurons; the increase in inward current at −120 mV was 0·43 ± 0·09 nA ($n = 5$).

Activation of single channels by arachidonic acid

To observe arachidonic acid-induced current at the single-channel level, inside-out patches from cell bodies were formed in symmetrical 140 mM KCl (reversal potential, 0 mV) and the fatty acid (10–20 μM) was applied to the cytoplasmic side of the membrane. Arachidonic acid elicited opening of an ion channel as shown in Fig. 3*A* (a hypothalamic cell) and this was observed in 23% (29/124; hypothalamic) and 20% (23/116; mesencephalic) of the patches tested, indicating a low densitiy of such channels in these neurons. Onset of activation was generally rapid and occurred within 10 s. Steady-state activation was observed within 30 s after application. Figure 3*B* shows activation of a channel in an outside-out patch from a mesencephalic neuron in which arachidonic acid was added to the extracellular side of the membrane. In outside-out patches, the time to onset of activation was slower and it took longer to reach a steady-state level, suggesting that the site of activation is probably at the cytosolic side of the membrane. These results were observed in seven mesencephalic and eight hypothalamic neurons. When arachidonic acid was applied only to the bath solution perfusing the membrane outside the pipette in the cell-attached state (Fig. 3*C*; mesencephalic neuron), the channels were also activated, albeit at a slower rate, indicating that the fatty acid could diffuse into the cell and reach the site of activation. Similar results to those shown in Fig. 3*C* were observed in six hypothalamic and five mesencephalic neurons.

Altogether we found three types of arachidonic acid-activated channel. The single channel openings at various membrane potentials, the open time durations and current–voltage (*I–V*) relationships in symmetrical 140 mM KCl medium for each of three types are shown in Figs 4–6 for mesencephalic neurons. Figure 4 shows a channel with a slightly outwardly rectifying *I–V* relationship. Figure 5 shows a channel with an inwardly rectifying *I–V* relationship. Figure 6 shows a channel with a relatively linear *I–V* relationship between −60 and +60 mV. Outside this range of membrane potentials, the increase in current amplitude for a given change in membrane potential was less, thus giving the *I–V* relationships a sigmoidal shape. Single channel slope conductances at +60 and −60 mV, mean slope times and burst durations are given in Table 1. Thus three types of channel with similar mean open times but different conductances were activated by arachidonic acid. Activation of all three types of ion channel probably gave rise to the whole-cell current observed in Fig. 2. Ion channels with kinetic properties indistinguishable from those in mesencephalic neurons were also found in hypothalamic neurons (Table 1).

The channels with linear and inwardly rectifying *I–V* relationships were activated more frequently than the channel with the outwardly rectifying *I–V* relationships in both types of neuron. Out of fifty-two patches in which arachidonic acid activated one of the channels described above, twenty-four, nineteen and nine patches showed channels with inwardly rectifying, linear and outwardly rectifying *I–V* relationships, respectively. In a few patches, simultaneous opening of two types of channel

was observed, indicating that different conductances activated by arachidonic acid are probably due to separate molecular entities.

Ion selectivity and voltage dependence

All three channel types were K^+-selective ion channels as judged by the following criteria. All solutions used contained 5 μM arachidonic acid to activate and maintain some activity of the channel being studied. K^+ channels were not open before addition of the fatty acid. In inside-out patches containing an inwardly rectifying channel, when [KCl] in the bath was changed from 140 to 70 or 28 mM, the reversal potential was shifted from 0 to 21 $\pm$ 2 or 44 $\pm$ 3 mV, respectively, close to the expected reversal

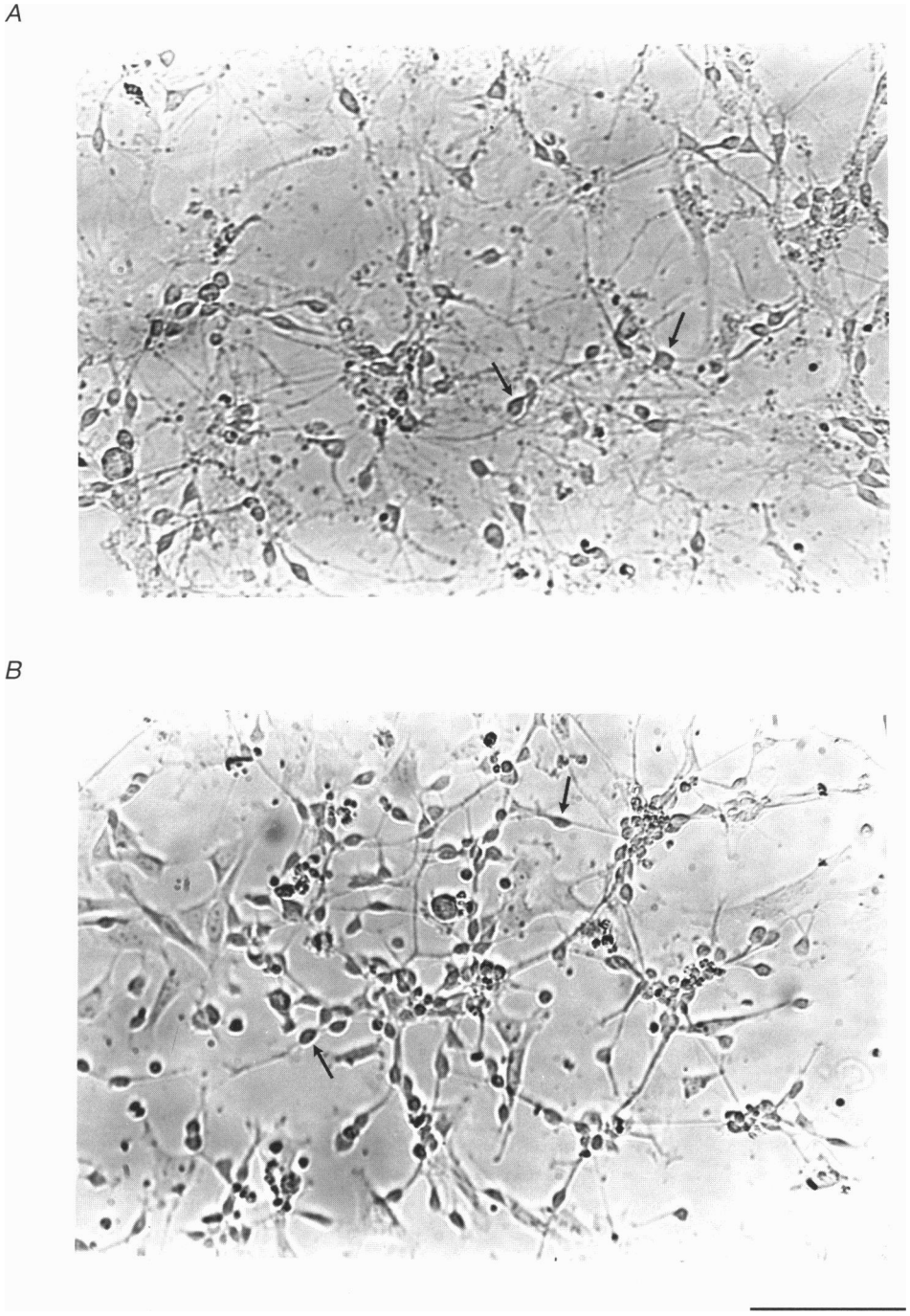

Figure 1. Photomicrographs of cultured neuronal cells viewed under phase contrast microscopy

A, mesencephalic neurons after 3 days in culture. *B*, hypothalamic neurons after 2 days in culture. Arrows indicate cell bodies which can be clearly identified. Scale bar, 100 μm.

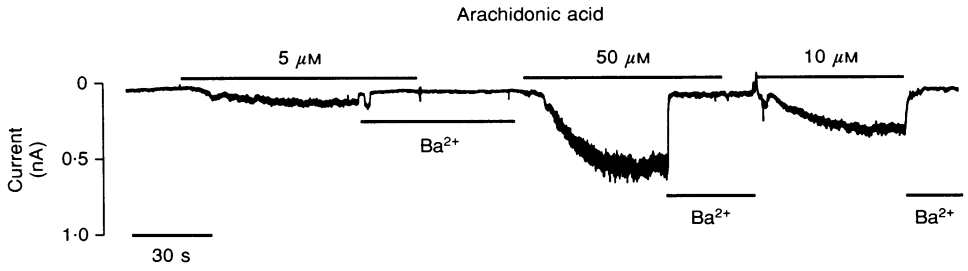

Figure 2. Activation of a whole-cell current by arachidonic acid in a mesencephalic neuron

Cell membrane potential was held at −60 mV relative to reversal potential to measure inward current. K^+ in bath solution was 10 mM. At steady state, solution containing arachidonic acid was applied extracellularly with or without 2 mM Ba^{2+}. Arachidonic acid was washed off before applying a different concentration of arachidonic acid. Arachidonic acid elicited a concentration-dependent activation of an inward current that was blocked by Ba^{2+}.

potentials for a K^+-selective channel ($n = 3$ each). For a Cl^- channel, the reversal potentials would be shifted to −17 and −40 mV, respectively. No measurable outward currents were observed between 0 and +80 mV when the cytosolic K^+ was replaced with equimolar amounts of Na^+, Cs^+, Li^+ or choline ($n = 4$ each). When K^+ in the pipette was replaced with one of the cations, and the channel activated by cytosolic solution containing 140 mM K^+ and arachidonic acid, only outward currents were present. Replacement of Cl^- with glutamate or

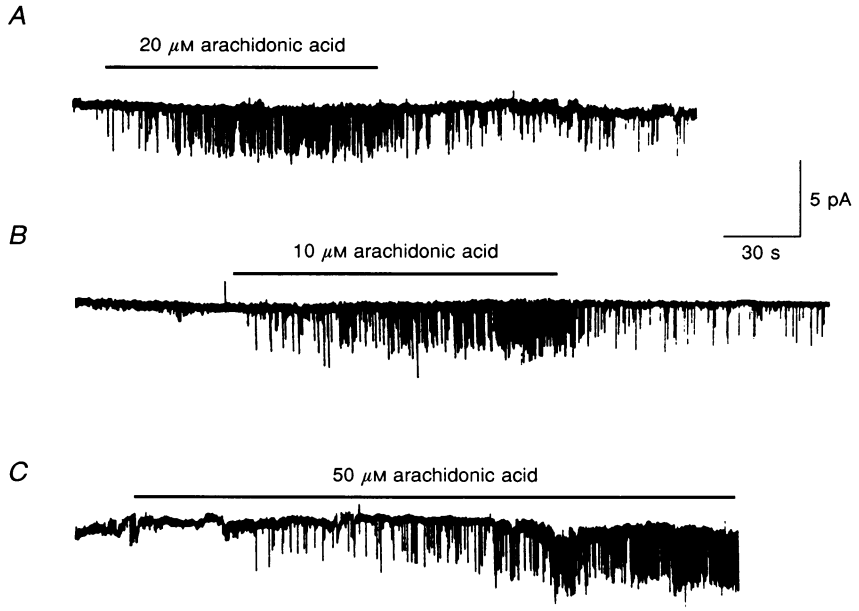

Figure 3. Activation of an ion channel by arachidonic acid

A, an inside-out patch was formed on the cell body of a hypothalamic neuron and arachidonic acid (20 μM) was applied slowly to the cytosolic face of the membrane. Channel openings were seen within a few seconds of application. Washout of the fatty acid resulted in closing of the channel. Similar results were observed in an additional 12 patches (6 with inwardly rectifying, 4 with linear and 2 with outwardly rectifying *I–V* relationships). *B*, an outside-out patch was formed on a mesencephalic neuron. Application of arachidonic acid (10 μM) to the extracellular side of the membrane caused a slow increase in channel opening that was also reversible. Similar results were observed in 7 mesencephalic neurons (4 with inwardly rectifying and 3 with linear *I–V* relationships) and 8 hypothalamic neurons (4 with inwardly rectifying, 3 with linear and 1 with outwardly rectifying *I–V* relationships). *C*, arachidonic acid was applied to the outside of the pipette in cell-attached patches. Slow onset of activation is apparent. Similar results were obtained in 5 mesencephalic neurons (3 with inwardly rectifying and 2 with linear *I–V* relationships) and 3 hypothalamic neurons (2 with inwardly rectifying and 1 with outwardly rectifying *I–V* relationships). Cell membrane potential was held at −30 mV. Pipette and bath solutions contained (mM): 140 KCl, 2 $MgCl_2$, 1 EGTA and 10 Hepes (reversal potential, 0 mV).

Table 1. Single channel properties of arachidonic acid-activated K^+ channels

	Mesencephalic neurons			Hypothalamic neurons		
Parameters	IR	L	OR	IR	L	OR
Conductance (pS)						
+60 mV	52 ± 6	45 ± 3	143 ± 12	54 ± 4	43 ± 3	152 ± 10
−60 mV	136 ± 11	47 ± 3	106 ± 9	138 ± 9	47 ± 5	110 ± 8
Mean open burst time (ms)	1·4 ± 0·2	1·2 ± 0·1	1·3 ± 0·2	1·3 ± 0·1	1·4 ± 0·1	1·2 ± 0·1
Burst duration (ms)	27 ± 7	36 ± 6	32 ± 5	32 ± 8	34 ± 4	29 ± 4

IR, inwardly rectifying; L, linear; OR, outwardly rectifying. All values were determined from 3 separate patches.

aspartate did not significantly shift the reversal potential (2 ± 3 mV shift) or change the *I–V* relationship, indicating that the channels were not permeable to Cl^-. These ion substitution studies were done for all three types of channel, and the results indicated that they were all K^+ selective. For channels with the outwardly rectifying and linear *I–V* relationships, the shifts in reversal potential produced by reducing the intracellular KCl concentration to 70 mM were 18 ± 2 and 19 ± 2 mV ($n = 3$ each) to the positive direction, respectively.

The following studies to identify potential inhibitors were done in twelve mesencephalic (four inside-out patches for each one of the three types of K^+ channel) and nine hypothalamic cells (three inside-out patches for each type). Single channel current amplitudes and channel activities were determined for each patch. All of the results described below except Ba^{2+} showed that the amplitude and channel activity did not change significantly (< ± 3% change; $P > 0.05$). Thus, arachidonic acid (50 μM)-activated K^+ channel currents

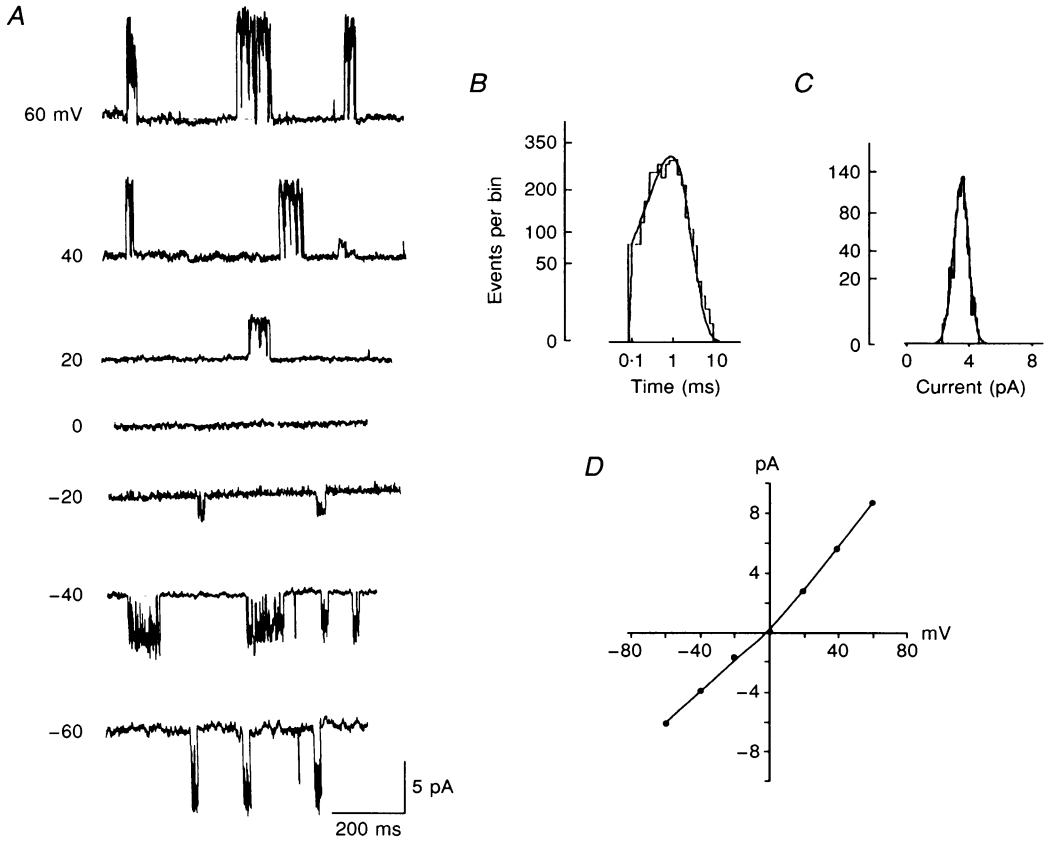

Figure 4. Single channel currents activated by arachidonic acid in an inside-out patch of a mesencephalic neuron in symmetrical 140 mM KCl

Arachidonic acid (20 μM) was applied to the cytosolic side of the membrane to elicit opening of one type of K^+ channel. Single channel amplitudes at various holding potentials were measured (*A*) and the *I–V* relationship was obtained (*D*). Note the slightly outwardly rectifying *I–V* relationship. The open time duration and amplitude histogram of channels open at −40 mV are shown in *B* and *C*, respectively.

were not blocked by 100 μM Ca^{2+} or 100 μM Gd^{3+} whether added to the pipette, bath or both. However, 2 mM Ba^{2+} inhibited channel openings (outward current at +60 mV) when added to the bath solution, providing additional evidence for the K^+-selective nature of the fatty acid-activated channels. The kinetic properties of K^+ channels were not affected by other known K^+ channel blockers such as 4-aminopyridine (5 mM), tetraethylammonium chloride (10 mM) and apamin (100 nM). Other ion channel modifiers such as nifedipine (10 μM), quinidine (100 μM), tetrodotoxin (10 μM), DIDS (10 μM), amiloride (1 mM) and glyburide (100 μM) produced no significant effect on channel activity and single channel conductance. Thus, the three types of K^+ channel possess distinct channel properties that are similar to the fatty acid-activated K^+ channels present in cardiac cells (Kim & Clapham, 1989), but different from any other known classes of K^+ channels.

In hypothalamic neurons, arachidonic acid activated the K^+ channels in cells treated with 200 μM indomethacin (an inhibitor of cyclo-oxygenase) and 10 μM nordihydroguaiaretic acid (NDGA; an inhibitor of lipoxygenase) for 15 min. Figure 7 shows the results of such experiments showing activation of three different types of K^+ channel by arachidonic acid in the presence of blockers ($n = 4$ each). Similar activation of K^+ channels with inwardly rectifying ($n = 4$) relationships were observed in mesencephalic neurons. These results indicate that the fatty acid itself can activate the K^+ channels.

The voltage dependence was studied for all three types of arachidonic acid-activated K^+ channel current in mesencephalic neurons. Inside-out patches were formed and K^+ channels activated with a low concentration of arachidonic acid (10 μM). When one of the K^+ channels was activated, membrane potentials were held at various levels (−60 to +60 mV) and channel activities calculated at each potential. Open probability was calculated and plotted as a function of membrane potential. Five determinations were made for each channel type. As shown in Fig. 8, the channels were generally more active at depolarized than at hyperpolarized potentials for the inwardly and outwardly rectifying channels. In contrast, the channel with the linear *I–V* relationship was relatively voltage independent, distinguishing it from the other two channel types.

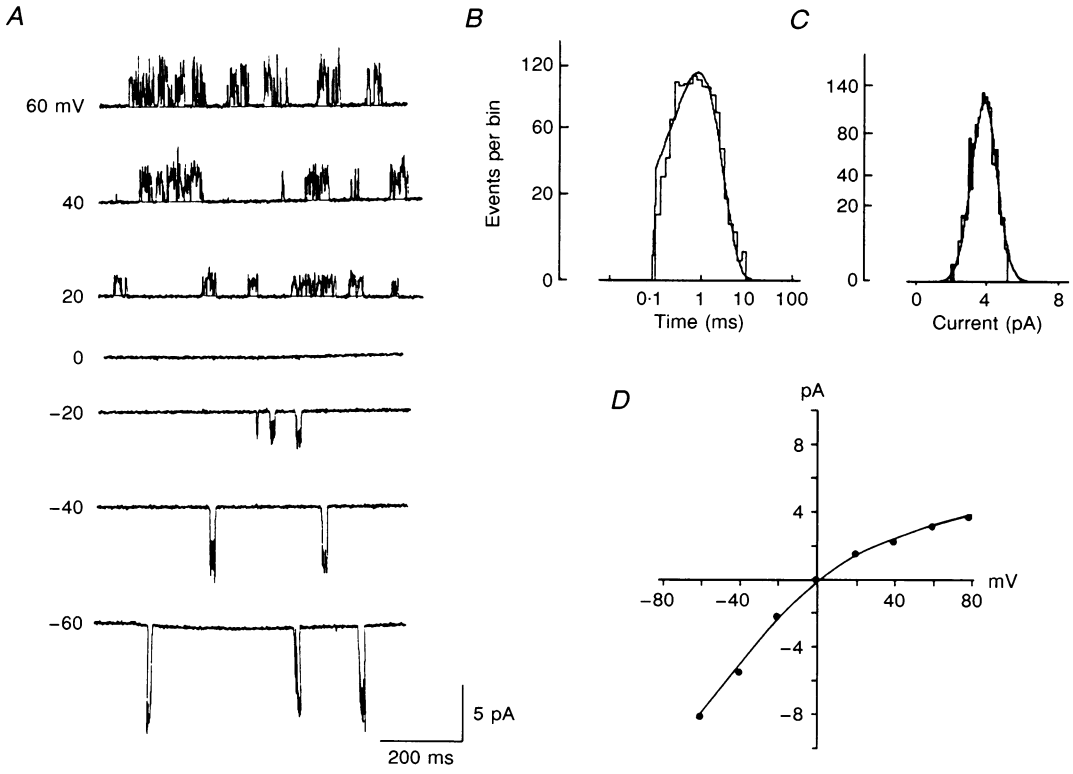

Figure 5. Single channel currents activated by arachidonic acid in an inside-out patch of a mesencephalic neuron in symmetrical 140 mM KCl

Arachidonic acid (10 μM) was applied to the cytosolic side of the membrane to elicit opening of a second type of K^+ channel. Single channel amplitudes at various holding potentials were measured (*A*) and the *I–V* relationship was obtained (*D*). Note the inwardly rectifying *I–V* relationship. The open time duration (at −40 mV) and amplitude (at −30 mV) histograms of channels open are shown in *B* and *C*, respectively.

Concentration and fatty acid dependence of K^+ channel activation

We initially used 10 or 20 μM arachidonic acid to study channel activation. To obtain the full concentration–effect relationship, the cytosolic surfaces of inside-out patches were exposed to increasing concentrations of arachidonic acid from 1 up to 100 μM. Generally, a K^+ channel started to open at 2 or 5 μM arachidonic acid, and the channel activity increased steeply from 5 to 50 μM. Near-maximal activation was usually observed at 50 μM arachidonic acid (Fig. 9). Changes in K^+ channel activity in response to increasing concentrations of arachidonic acid in an inside-out patch from a mesencephalic neuron is illustrated in Fig. 9*B*. Three inwardly rectifying channels were present in this patch. Channel open probability averaged every second is shown in Fig. 9*A* and the results from five such experiments are shown in Fig. 9*C*. The open probability was calculated by integrating the current through all channels divided by the total current that would pass through all the channels if they were fully open. The total number of channels in a given patch was estimated by the number of channels open after application of 200 μM arachidonic acid and 40 mmHg pressure. Data points were fitted by linear regression to the Hill equation:

$$P_o = P_{o(m)}/[1 + (K_{1/2}/[AA])^n],$$

where $P_{o(m)}$ is the observed maximum P_o, $K_{1/2}$ (22 ± 4 μM) is the concentration of arachidonic acid at which half-maximal activation occurs, AA refers to arachidonic acid, and n (= 1·1) is the Hill coefficient. These results indicate that arachidonic acid produces a concentration-dependent activation of the K^+ channel, in a manner similar to that observed for a one-to-one ligand–receptor interaction. For the other two types of K^+ channel, we used 20 μM arachidonic acid to determine the degree of activation. The results showed that this concentration of arachidonic acid activated 54 ± 12 and 43 ± 8% ($n = 4$) of the maximum observed with 100 μM for the channels with linear and outwardly rectifying *I–V* relationships, respectively. Although similar analyses of concentration-dependent effects of arachidonic acid were not done in hypothalamic neurons, K^+ channels also responded to 20 μM arachidonic acid in a manner similar to that observed in mesencephalic neurons in five patches.

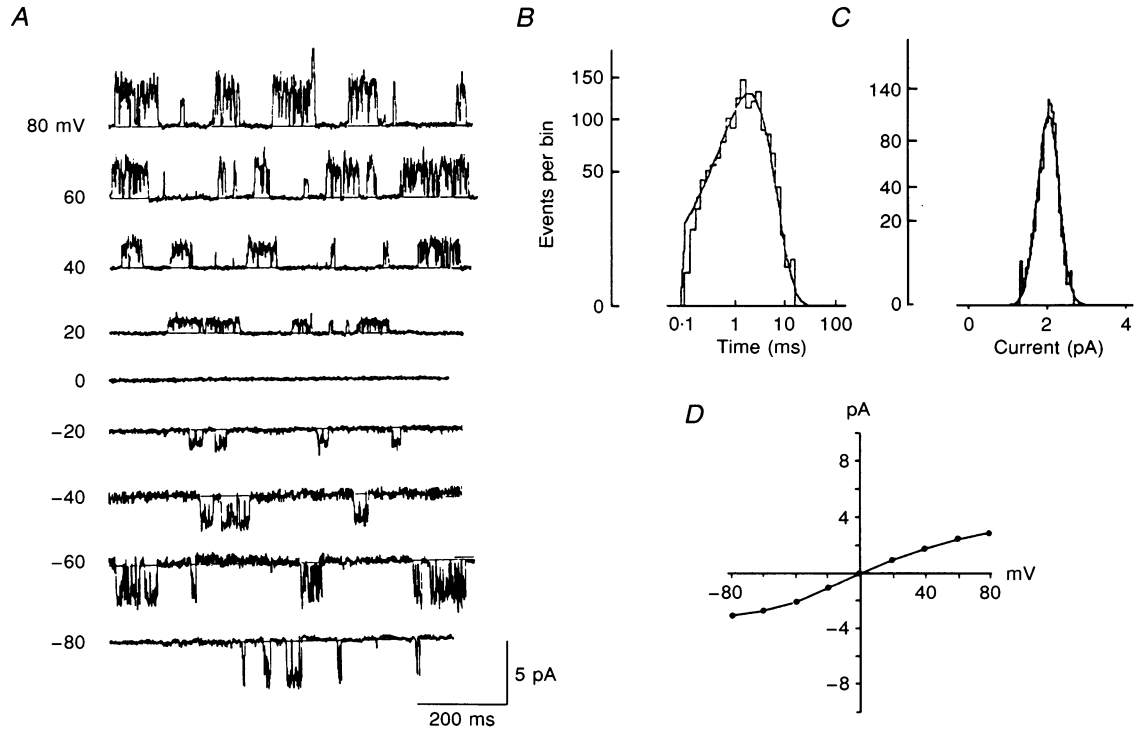

Figure 6. Single channel currents activated by arachidonic acid in an inside-out patch of a mesencephalic neuron in symmetrical 140 mM KCl

Arachidonic acid (10 μM) was applied to the cytosolic side of the membrane to elicit opening of a third type of K^+ channel. Single channel amplitudes at various holding potentials were measured (*A*) and a *I–V* relationship was obtained (*D*). Note the linear *I–V* relationship. The open time duration and amplitude histograms of channels open at −40 mV are shown in *B* and *C*, respectively.

To test the effectiveness of other free fatty acids in K^+ channel activation, inside-out patches were perfused with various types of saturated and unsaturated free fatty acids. Figures 10 and 11 show the effect of free fatty acids on a mesencephalic and a hypothalamic neuron, respectively. The patches used for these experiments were chosen such that arachidonic acid clearly and rapidly activated the channels in a reversible way. Therefore, if a free fatty acid did not activate a K^+ channel in the same patch, it was taken as an inactive compound. Each patch was tested for channel activation by several fatty acids, and the results were judged to be satisfactory if one of the known activators of the K^+ channel (for example, arachidonic acid) was still able to elicit channel opening at the end of the experiment. This provided proof that the channels were not desensitized or run down. All free fatty acids were tested up to 200 μM. Of the fatty acids tested, only arachidonic, linoleic, linolenic and docosahexaenoic acids were capable of activating one of the three types of K^+ channel in both types of neuronal cell culture. The activation by a free fatty acid could be repeated many times after washout in between applications, and the extent of activation remained relatively unchanged with time. In general, we did not expose the patch membrane to an activating fatty acid for more than 3 min since longer exposures required longer time to wash out the effect. Saturated and mono-unsaturated free fatty acids such as palmitic, stearic, arachidic, palmitoleic, oleic and elaidic acids did not activate a K^+ channel. Like arachidonic acid, linoleic, linolenic and docosahexaenoic acids could activate all three types of K^+ channel. A summary of these results is shown in Table 2. Since the activation profile was identical in both hypothalamic and mesencephalic neurons, a fatty acid that activated any one of the three K^+ channel types in either type of neuron was taken as an active compound. Interestingly, arachidonoyl alcohol and arachidonic acid methyl ester failed to activate the K^+ channel, suggesting that the charged carboxyl group of the fatty acid is an important site of interaction. Thus, there appears to be certain structural specificities to channel activation by free fatty acids.

Mechanosensitivity of the K^+ channel

To test the hypothesis that the arachidonic acid-activated K^+ channel in neurons is also a stretch-activated channel, cell-attached patches were formed and negative pressure applied to the inside of the pipette. Application of negative pressure (0–40 mmHg) elicited opening of a

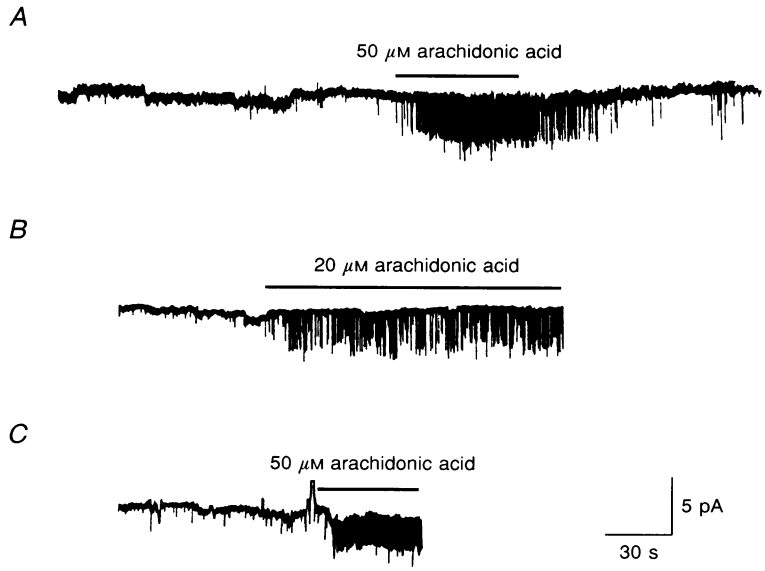

Figure 7. Activation of K^+ channels by arachidonic acid in cells treated with indomethacin and NDGA in hypothalamic cells

Cells were incubated with 200 μM indomethacin and 10 μM NDGA for 15 min to block enzymes that metabolize arachidonic acid. Inside-out patches were formed from these cells in the presence of inhibitors and then arachidonic acid was applied to the cytoplasmic side of the membrane. K^+ channels with inwardly rectifying (*A*), outwardly rectifying (*B*) and linear (*C*) relationships are shown. The type of K^+ channel in each patch was determined at the end of each experiment by determining the current amplitude at different membrane potentials (−60 to +60 mV). Pipette and bath solutions contained 140 mM KCl (reversal potential, 0 mV). The cell membrane potentials in patches shown in *A*, *B* and *C* were −30, −30 and −60 mV, respectively.

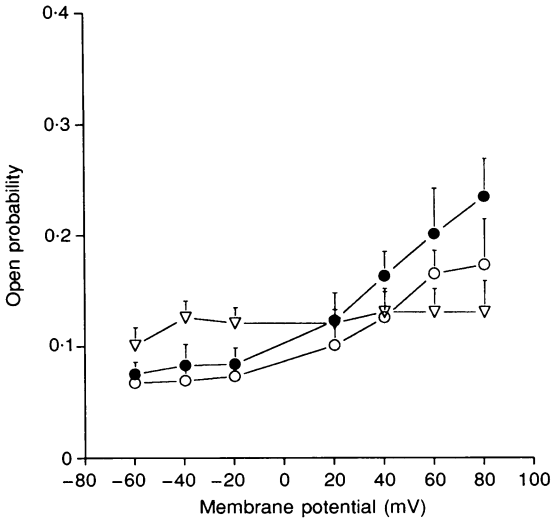

Figure 8. Voltage dependence of channel open probability of three types of K^+ channel

Inside-out patches were formed on the cell body of mesencephalic neurons and arachidonic acid (10 μM) was applied to the internal side of the membrane to activate one of the three types of K^+ channel. At steady state (after ~3 min), membrane potentials were changed from −60 to +80 mV at 20 mV intervals, and channel open probability calculated at each potential. Each point is the mean of 5 determinations from 5 separate patches and vertical bars represent S.E.M. The channels with outwardly rectifying (●), inwardly rectifying (○) and linear (▽) I–V relationships are shown.

channel with kinetic properties (i.e. I–V relationships, mean open times, burst durations and insensitivity to all drugs tested except Ba^{2+}) identical to those seen on activation by arachidonic acid. The channels were K^+ selective, as judged by the same set of criteria used for the fatty acid-activated channel. The channel shown in Fig. 12*A* was an outwardly rectifying type from a hypothalamic neuron; however, the inwardly rectifying and non-rectifying channel currents were also sensitive to pressure in a similar way. The pressure dependence of channel activation can be clearly seen in Fig. 12*B* in which time-averaged open probability is plotted. The

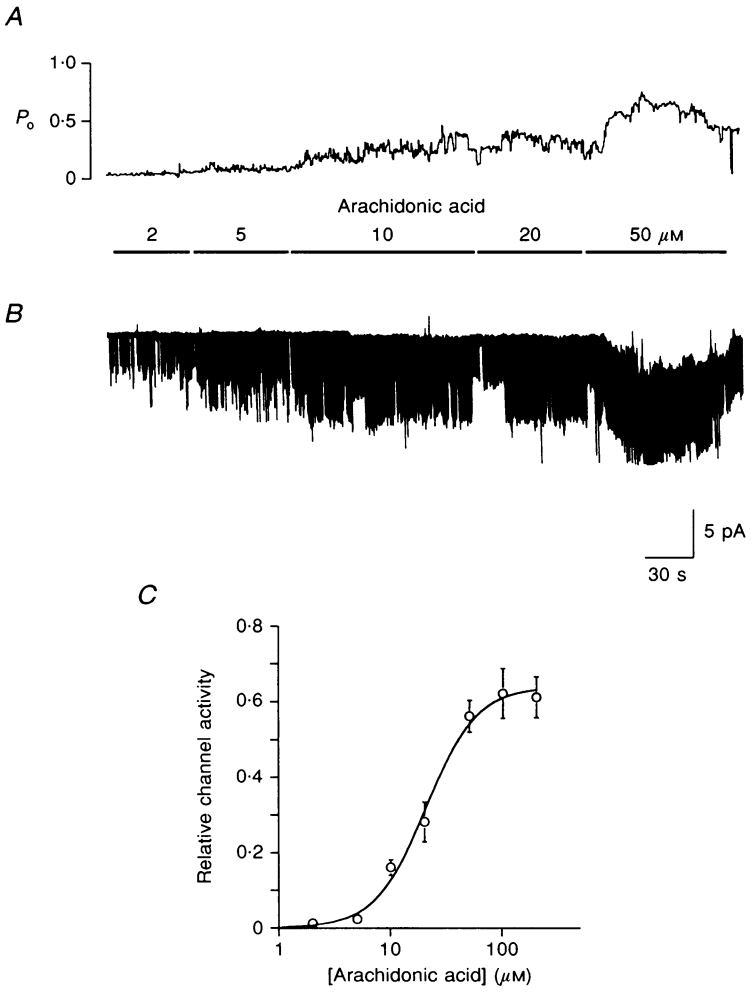

Figure 9. Concentration dependence of channel open probability

Inside-out patches were formed on the cell body of a mesencephalic neuron and arachidonic acid was applied to the internal surface at increasing concentrations (*B*). The K^+ channel shown here was of the inwardly rectifying type. Membrane potential was held at −40 mV. Channel open probability was averaged every second and plotted as a function of time (*A*). Open probability was measured at each concentration of the fatty acid and plotted as a function of fatty acid concentration (*C*). Each point is the mean of 5 determinations from 5 separate patches with the inwardly rectifying channel. Points were fitted by the least-squares method to the Hill equation: $P_o = P_{o(m)}/[1 + (K_{½}/[AA])^n]$, where P_o is open probability, AA is arachidonic acid, $K_{½}$ is concentration at which half-maximal effect is seen (22 ± 4 μM), observed maximum $P_{o(m)}$ is 0·68, and n is the Hill coefficient (1·1).

channel activation by pressure was reversible and could be repeated many times in the same patch. No desensitization of channel opening was observed when the negative pressure was held constant for more than 3 min. Such stretch-activated channels were observed in both mesencephalic and hypothalamic neurons. For example, Fig. 12*C* shows an inwardly rectifying channel current in a mesencephalic neuron, showing the activation of a K^+ channel by pressure application. Open probability as a function of applied pressure is shown in Fig. 12*D* for the three types of K^+ channel in hypothalamic neurons. Data points were fitted by linear regression to a modified Boltzmann distribution given by:

$$P_o = \{\exp[(p - p_{½})/s]\}/\{1 + \exp[(p - p_{½})/s]\},$$

where P_o is the opening probability, p is the negative pressure, $p_{½}$ is the pressure at which P_o is 0·5, and s is the slope of the plot of $\ln[P_o/(1 - P_o)]$ *vs.* pressure. $p_{½}$ was 18 ± 3 mmHg ($n = 3$). An e-fold change in activity (ratio of probability of being open to probability of being closed) occurred for every 5·0 mmHg change in pressure. For the inwardly rectifying and non-rectifying channels, the pressures to produce half-maximal effects were 18 and 16 mmHg, respectively ($n = 3$). None of the three types of K^+ channel were blocked by 100 μM $GdCl_3$, an inhibitor of certain stretch-activated ion channels (Yang & Sachs, 1989) or by 100 μM glibenclamide, an inhibitor of the ATP-sensitive K^+ channel (Nichols & Lederer, 1991). Applying albumin (2 mg ml^{-1}) to the inside-out patches to remove free fatty acids that may be released by stretch failed to block K^+ channel activation by pressure application for all three K^+ channels in hypothalamic neurons ($n = 3$ each).

Since two separate manoeuvres (application of a fatty acid and stretch) caused activation of the same channel, we examined whether arachidonic acid alters the sensitivity of the channel to membrane stretch. The cytoplasmic face of inside-out patches from a mesencephalic neuron was first perfused with 5 μM arachidonic acid to maintain a low activity of a K^+ channel (Fig. 13). Then, 50 μM arachidonic acid was applied to check for the response of the channel. Arachidonic acid always caused marked activation of the K^+ channels. Reduction of the fatty acid concentration to 5 μM gradually decreased the channel activity to the initial level. To the same patch, desired magnitudes of negative pressure were applied to the patch. In the example shown in Fig. 13*A* and *B*, applying a negative pressure (26 mmHg) to the pipette resulted in an increase

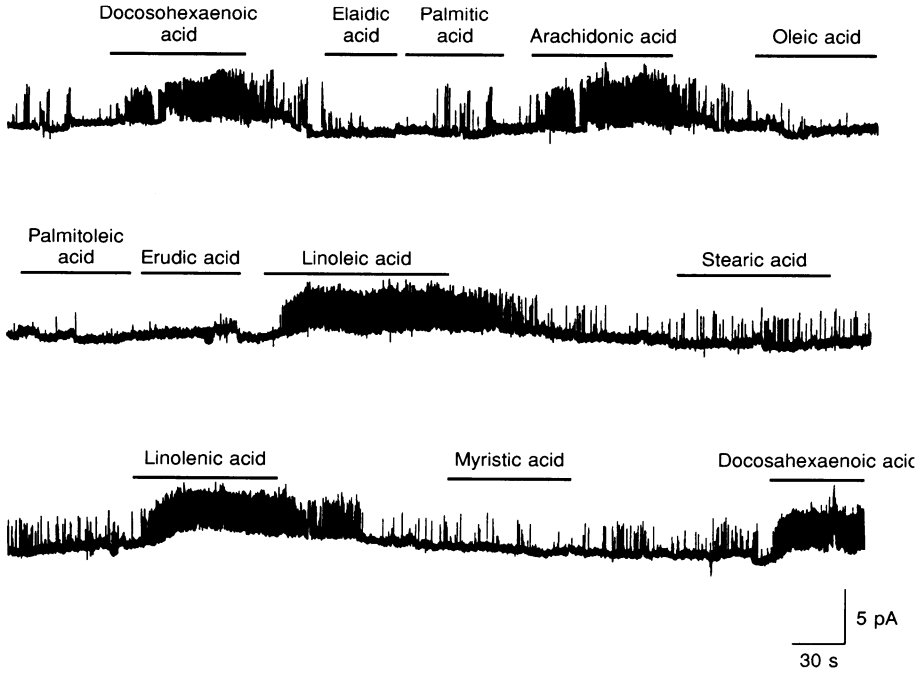

Figure 10. Activation of a K^+ channel with an outwardly rectifying *I–V* relationship in an inside-out patch of a mesencephalic neuron by free fatty acids

Cell membrane potential was initially held at +40 mV (top tracing) and changed to +30 mV (lower 2 tracings) to help maintain the patch for a longer period of time. Fatty acids (50 μM) were applied sequentially to the cytosolic side of the membrane to determine which fatty acids caused activation of the K^+ channel. A continuous current tracing obtained from 1 inside-out patch is shown. In this example, linolenic, linoleic, docosahexaenoic and arachidonic acids were potent activators of the K^+ channel. In other patches, fatty acids were applied in different sequences to determine accurately which fatty acid was effective. In all patches, 1 of the 4 fatty acids listed above was applied at the end of an experiment to check for the ability of the channel to respond.

Table 2. Ability of various compounds to activate the neuronal K^+ channel current

Compound	Abbreviations	Bond Positions	No. activated/ no. with a K^+ channel
Myristic acid	(14:0)		0/12 (5, 5, 2)
Palmitic acid	(16:0)		0/12 (4, 6, 2)
Palmitoleic acid	(16:1[n-7])	Δ9	0/8 (4, 4, 0)
Stearic acid	(18:0)		0/8 (3, 4, 1)
Oleic acid	(18:1[n-9])	Δ9	0/12 (5, 6, 1)
Petroselenic acid	(18:1[n-12])	Δ6	0/6 (3, 3, 0)
Elaidic acid	(trans-18:1[n-9])	t-Δ9	0/12 (6, 4, 2)
Linoleic acid	(18:2[n-6])	Δ9, 12	18/18 (8, 8, 2)
γ-Linolenic acid	(18:3[n-6])	Δ6, 9, 12	22/22 (10, 8, 4)
Arachidic acid	(20:0)		0/11 (5, 5, 1)
Arachidonic acid	(20:4[n-6])	Δ5, 8, 11, 14	52/52 (24, 19, 9)
Erucic acid	(22:1[n-9])	Δ13	0/12 (5, 4, 3)
Docosahexaenoic acid	(22:6[n-3])	Δ4, 7, 10, 13, 16, 19	16/16 (7, 6, 3)
Nervonic acid	(24:1[n-9])	Δ15	0/8 (4, 4, 0)
Arachidonyl alcohol			0/7 (5, 2, 0)
Arachidonic acid methyl ester			0/6 (3, 3, 0)

Only patches in which one or more of the three types of K^+ channel were present as judged by its activation by arachidonic acid or application of negative pressure were included. In the nomenclature of fatty acids: n indicates the position of the first double bond from the methyl end (IUPAC-IUB Commission on Nomenclature); Δ describes double bond positions relative to the carboxyl carbon of the acyl chain. The three numbers in parentheses in the last column indicate the number of patches showing K^+ channels with inwardly rectifying, linear and outwardly rectifying *I–V* relationships, respectively.

in channel activity similar to that produced by 50 μM arachidonic acid. A lower negative pressure (12 mmHg) caused a smaller increase in channel activity. The pressure at which half-maximal activation occurred (relative to basal) was 17 ± 3 mmHg ($n = 3$), and e-fold change in activity occurred for every 5·3 mmHg change in pressure. Thus, the sensitivities of the channel to membrane stretch in the presence or absence of

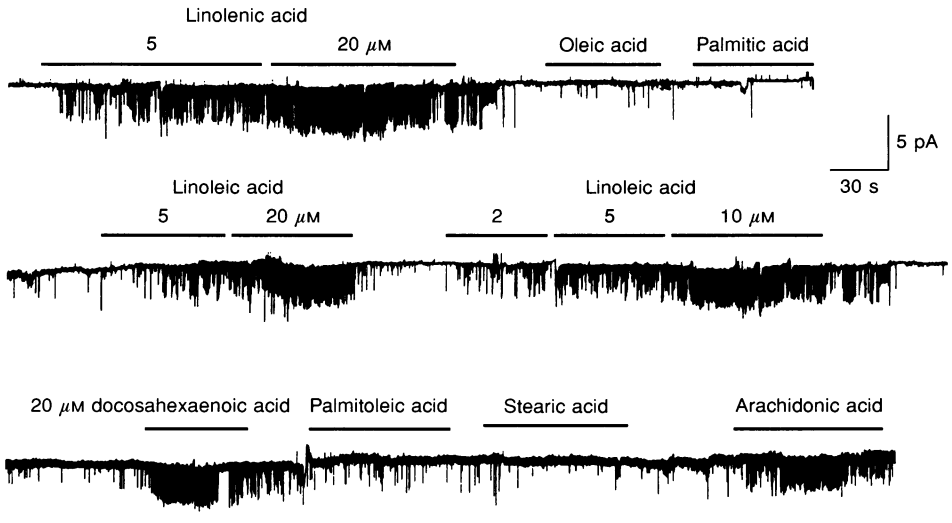

Figure 11. Activation of a K^+ channel with an inwardly rectifying *I–V* relationship in a hypothalamic neuron by free fatty acids

Cell membrane potential was held at −30 mV. Fatty acids were applied sequentially to the cytosolic side of the membrane. A continuous current tracing obtained from 1 inside-out patch is shown. In this example, linolenic, linoleic, docosahexaenoic and arachidonic acids were also the activators of the K^+ channel. In other patches, application of fatty acids in different sequences also showed that the 4 fatty acids were the only species tested that were effective. In all patches, 1 of the 4 fatty acids listed above was applied at the end of an experiment to check for the ability of the channel to respond. Concentration of fatty acids other than those shown was 100 μM in this tracing.

arachidonic acid were similar. To be certain that arachidonic acid and pressure indeed activated identical K^+ channels, near-maximal activation of the K^+ channel was induced separately in an inside-out patch with either 50 μM arachidonic acid or 25 mmHg pressure. Figure 13*C* shows a patch from a hypothalamic neuron where only one K^+ channel was present. When both arachidonic acid and pressure were applied at the same time to the patch, only one K^+ channel was activated, showing that the two manoeuvres do not have additive effects when open probability is high, and therefore indicating that the same K^+ channel was involved. Under these experimental conditions, two channel openings would be observed by application of both stimuli if the K^+ channels were from separate populations. Similar results were observed in three other hypothalamic neurons (two inwardly rectifying and two non-rectifying channels) and in five mesencephalic neurons (three inwardly rectifying and two outwardly rectifying channels).

Effect of pH on channel activity

It has been shown previously that the arachidonic acid-activated K^+ channels in cardiac cells are activated by a reduction in intracellular pH (Kim, 1992). To study whether the neuronal K^+ channels possess such a property, the effect of pH on K^+ channel activity was measured. Inside-out patches were exposed to 5 μM arachidonic acid to cause a small activation of one of the three types of K^+ channel (at the arrow in Fig. 14*A*). Figure 14 shows a patch with an inwardly rectifying property in a hypothalamic neuron. The pH of the medium perfusing the cytosolic surface of inside-out patches was progressively changed from a control value of 7·2 to 5·6 in several steps. As shown in Fig. 14*A*, decreasing the pH of the medium caused a progressive increase in channel activity and this was readily reversible. The change in channel activity was due to an increase in the frequency of channel opening. No effect of pH (6·0–7·6) on single channel conductance was present. Increasing the pH from 7·2 to 7·4 or 7·6 reduced the channel activity only slightly (< 10%), indicating that the K^+ channel was more sensitive to pH levels in the acidic than alkaline range. Channel activity observed at pH 5·8 was taken as 1·0 (open probability at pH 5·8 was 0·32 ± 018, $n = 5$) and relative channel activities obtained from five inside-out patches containing inwardly rectifying channels were averaged and plotted as a function of pH (Fig. 14*C*). Half-maximal activation of the channel was observed at pH 6·4. When the K^+ channel was activated by linoleic or docosahexaenoic acid, reducing the pH of the medium from 7·2 to 6·4 led to a 3·02 ± 0·7-fold ($n = 3$) increase in channel activity in hypothalamic neurons. At a given level of negative

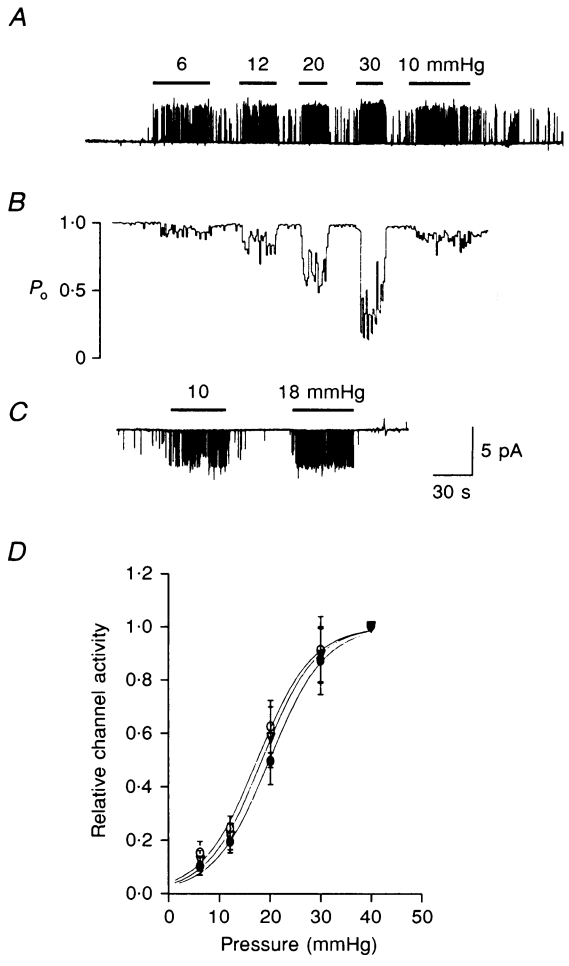

Figure 12. Activation of the K^+ channel by pressure

A cell-attached patch was formed on the cell body of a hypothalamic neuron. The K^+ channel was of the outwardly rectifying type. Cell membrane potential was held at +30 mV. Negative pressure applied to the inside of the pipette caused reversible pressure-dependent activation of the K^+ channel (*A*). The changes in channel open probability averaged every second is illustrated more clearly in *B*. A cell-attached patch from a mesencephalic neuron held at −30 mV also shows opening of an inwardly rectifying K^+ channel in response to negative pressure in the pipette (*C*). Relative open probability is plotted as a function of pressure in the pipette (*D*). Each point is the mean of 3 determinations from 3 patches (hypothalamic neurons) each containing an inwardly rectifying K^+ channel (●), an outwardly rectifying channel (▽) and a non-rectifying channel (○), and is fitted to a modified Boltzmann distribution as described in the text. The pressure at which half-maximal effect occurred ranged from 17 to 19 mmHg ($n = 3$).

pressure which caused a sustained activation of a K^+ channel, reducing the pH of the medium also increased channel activity, indicating that the concentration of H^+ itself and not the protonated form of free fatty acids is important in the modulation of channel activity. Although not shown here, the stimulatory effects of acidic pH (by changing pH from 7·2 to 6·4) on channel activity were observed for all K^+ channels in both hypothalamic ($n = 9$) and mesencephalic neurons ($n = 8$).

Lack of K^+ channel activation by glutamate

It has been reported that glutamate causes release of arachidonic acid via NMDA receptor activation and subsequent elevation of intracellular [Ca^{2+}] (Dumuis, Pin, Oomagari, Sebben & Bockaert, 1990; Miller *et al.* 1992). To test whether glutamate can cause activation of K^+ channels described in this study via endogenous formation of arachidonic acid, 100 μM glutamate was applied to the pipette and extracellular solutions in cell-attached patches in which 1 mM $CaCl_2$ and 10 μM glycine were also present. In eighteen mesencephalic and sixteen hypothalamic neuronal patches that possessed one of the K^+ channels, glutamate failed to activate the K^+ channels at least for the duration of the experiment (~10 min). Direct application of 10 μM Ca^{2+} to the cytoplasmic side of the membrane also failed to activate any K^+ channels in twelve neuronal patches (six of each cell type) despite the presence of a K^+ channel in the patch as judged by its activation by application of pressure or 50 μM arachidonic acid. Under similar experimental conditions, we failed to observe activation of a K^+ channel in nine neurons (five mesencephalic, four hypothalamic) with dopamine (100 μM), another neurotransmitter reported to generate arachidonic acid (Piomelli *et al.* 1991). Thus, the K^+ channels described in this study were not activated by glutamate and dopamine receptor-mediated pathways under the experimental conditions of this study. Therefore, the roles of the K^+ channels in receptor-mediated signal transduction pathways remain to be elucidated.

DISCUSSION

We have used primary cultures of neuronal cells prepared from either the mesencephalic or hypothalamic areas of fetal rat brain to study whether free fatty acids can

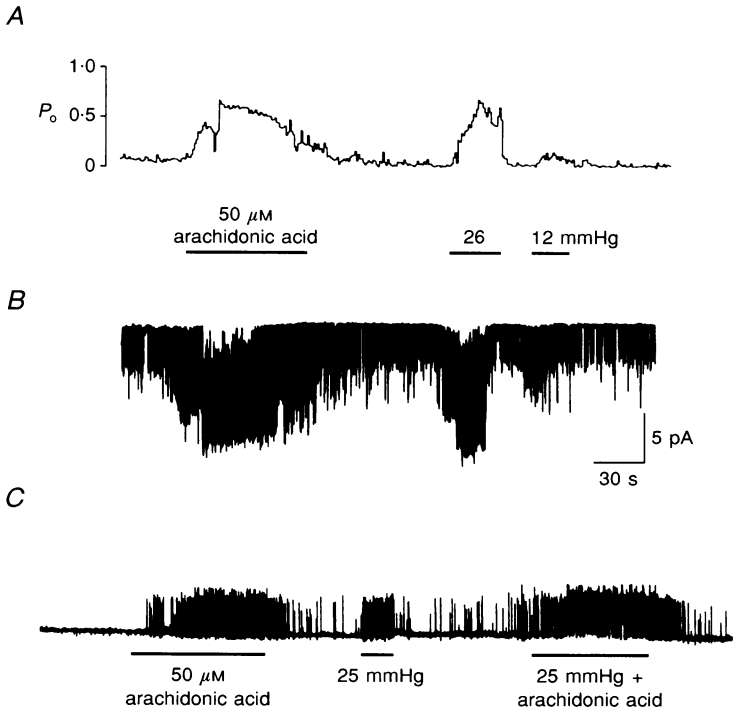

Figure 13. Activation of a K^+ channel in a mesencephalic neuron by arachidonic acid and pressure

Cell membrane potential was held at −35 mV. An inside-out patch was formed and 5 μM arachidonic acid was applied to the bath solution to activate a K^+ channel and was present for the entire experiment (*B*). The channel was of the inwardly rectifying type. A higher concentration of arachidonic acid (50 μM) was applied for ~1 min and then washed out. In the same patch, negative pressures were applied to the pipette, resulting in activation of the same K^+ channel. Changes in open probability averaged every second are shown in *A*. To observe possible additive effects when open probability is high, 50 μM arachidonic acid and 25 mmHg pressure were applied separately or together in the same inside-out patches of hypothalamic neurons (*C*). No additive effects were observed, indicating that the same K^+ channel was activated by both manoeuvres.

activate K^+-selective ion channels in neurons. In mesencephalic cells we identified three types of K^+ channel that are normally closed but can be activated by arachidonic acid applied to the extracellular or cytosolic side of the membrane. Subsequently, the three types of K^+ channel, with *I–V* relationhips, single channel conductances and mean open times indistinguishable from those in mesencephalic neurons, were also found in hypothalamic neurons. The kinetic properties of these channels and their sensitivity to pH and stretch are similar to those previously reported for fatty acid-activated K^+ channels in cardiac cells. In cardiac myocytes, the arachidonic acid-activated K^+ channel currents were of two types, an outwardly rectifying and a non-rectifying current (Kim, 1990; Wallert *et al.* 1991). In rat brain neurons used here, an additional inwardly rectifying current was also activated by fatty acids.

The mechanism by which free fatty acids activate a K^+ channel is not known. The results obtained thus far in cardiac muscle, smooth muscle and neuronal cells are consistent with the view that fatty acids act directly on the channel itself or an associated regulatory protein (Ordway, Singer & Walsh, 1991). Evidence that supports the direct action of fatty acids is that (1) inhibitors of cyclo-oxygenase (indomethacin) and lipoxygenase (NDGA) do not prevent activation of K^+ channels by free fatty acids, and (2) activation occurs in excised inside-out patches in which cytosolic components such as nucleotides and soluble second messengers are not present. A specific fatty acid binding site may be present in these K^+ channels, analogous to fatty acid binding domains in fatty acid or lipid binding proteins. A putative fatty acid binding domain in the NMDA receptor has also been described (Petrou, Ordway, Singer & Walsh, 1993).

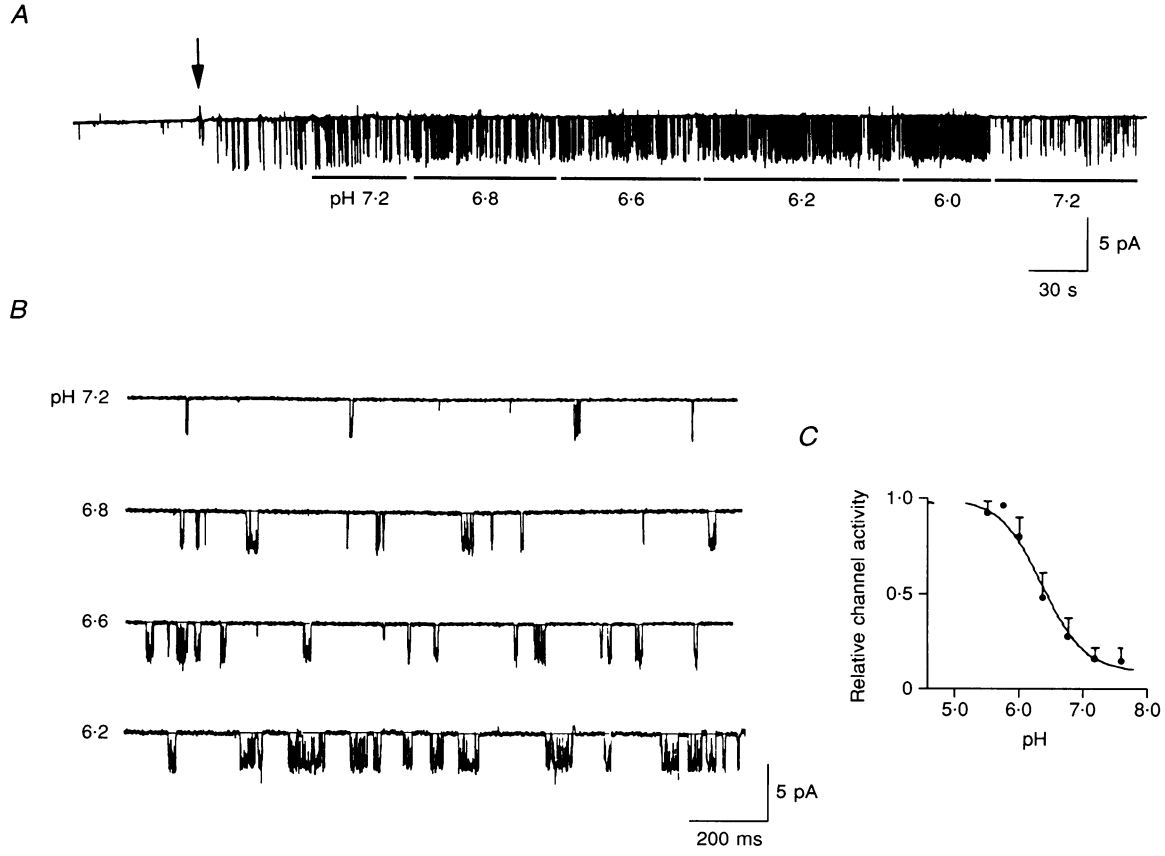

Figure 14. Effect of pH on the K^+ channel activity

Inside-out patches from hypothalamic neurons were exposed to 5 μM arachidonic acid to activate a K^+ channel (see arrow). The K^+ channel shown here was of the inwardly rectifying type (cell membrane potential, −30 mV). When steady-state activation was present, the pH of the internal solution was changed sequentially from 7·2 (control) to 5·6 and back to 7·2 (*A*). Changes in channel opening frequency can be discerned more easily in the expanded tracing (*B*). Plot of relative channel activity against pH is shown in *C*. Channel activity at pH 5·8 was taken as 1·0 ($n = 5$). Data points were fitted by linear regression to the equation:

$$y = 0{\cdot}1 + 1/[1 + ([H^+]/K_{½})],$$

where y is relative channel activity and $K_{½}$ (pH 6·3) is the $[H^+]$ at which half-maximal activation was present.

Studies with different classes of free fatty acids (Table 2) suggest that only certain polyunsaturated fatty acids are effective in channel activation. Since the carboxyl group seems important for activation, at least two separate domains on the free fatty acid may be required to cause activation: the carboxyl domain and the hydrophobic domain with double bonds. In addition to the specific sites in the structure of fatty acids, different forms of a given fatty acid in solution may need to be considered. For example, at low concentrations (5–20 μM), arachidonic acid in solution is expected to be in free acid form. Thus, when such low concentrations are used, it is expected that free acid causes the K^+ channel activation. The observation that channel activation increases further with higher concentrations of acrachidonic acid (50 μM), which are likely to contain free as well as micelle globules, suggests that perhaps micelles could also be exerting some effects on the channel. These possibilities are difficult to distinguish without knowing how different forms of arachidonic acid interact with the lipid–protein bilayer.

The finding that the fatty acid-activated K^+ channels are stretch sensitive suggests that tension also gates the channel opening. This raises the interesting question of whether fatty acids activate the channel by generating tension in the membrane or whether stretch activates the channel by generating free fatty acids from the membrane phospholipids. It is possible that free fatty acids insert into the leaflets of the phospholipid bilayer and generate enough local tension to activate a K^+ channel. However, this seems unlikely since, if such were the case, one would predict that other long chain fatty acids, both saturated and unsaturated, would also produce tension and activate the channel in a way similar to arachidonic, linoleic or linolenic acid. It also seems unlikely that stretch caused release of free fatty acids since albumin, a fatty acid binding protein, failed to block activation of the channel by stretch. The logical conclusion is that free fatty acids and tension are able to gate the channel separately, presumably involving different channel domains as the effects of two stimuli are additive. The stretch-induced activation of the channel may occur via a mechanism similar to that in *Escherichia coli* cell envelope in which the channels are gated by tension transduced via the lipid bilayer (Sukharev, Martinac, Arshavsky & Kung, 1993).

Whatever the mechanism, the effects of fatty acids on the activation of the K^+ channels in neurons were always consistent. As shown in Table 2, among the fatty acids tested, the ones that caused channel activation were arachidonic, linoleic, linolenic and docosahexaenoic acids. The activation profile in neurons differs from that in cardiac and smooth muscle cells, in which other classes of unsaturated and certain saturated fatty acids were also effective in channel activation. For example, oleic and myristic acids were able to activate K^+ channels in smooth muscle cells whereas they were ineffective in neuronal cells. This suggests that the fatty acid binding sites on the channel protein, if such sites exist, recognize different classes of fatty acids depending on the tissue type. The lack of effect of arachidonoyl alcohol and arachidonic acid methyl ester indicates that the carboxyl group of the arachidonic acid plays an important role in channel activation and also supports the idea that a specific binding site for fatty acid may be present in the channel protein.

The primary dispersed cultures used in these studies contain a heterogeneous population of neurons. The mesencephalic cultures are prepared from tissue containing the developing A8–A10 dopamine cell groups, but tyrosine hydroxylase-positive neurons represent up to 30% of the neurons in these cultures (Collier, Gallagher & Sladek, 1993). The remaining neurons represent γ-aminobutyric (GABA)-producing neurons (Masuko, Nakajima & Nakajima, 1992) and other unidentified neuronal phenotypes. The hypothalamic cultures also contain a variety of neuronal phenotypes, including neurons producing the hypothalamic hormones, GABA and dopamine (Madarasz, Kornyei, Poulain & Theodosis, 1992; Sladek & Gallagher, 1993). Therefore, we are not sure whether a subpopulation of neurons possess these K^+ channels or whether K^+ channels are present in all types of neuron. In support of the latter view, K^+ channels with similar kinetics and sensitivity to membrane stretch were also found in cell bodies of hippocampal and dorsal root ganglion neurons in rat (D. Kim, unpublished results). In both mesencephalic and hypothalamic neurons, we observed background inwardly rectifying K^+ channels that were open under basal conditions, Ca^{2+}-activated K^+ channels whose activity increased when Ca^{2+} was applied to the cytosolic medium and ATP-sensitive K^+ channels which were blocked by applying 2 mM ATP to the cytosolic medium. These channels were not activated by free fatty acids or application of negative pressure to the pipette.

K^+ channels play a fundamental role in neuronal cell excitability. They are involved in bringing the cell membrane potential to a more hyperpolarized state and in reducing the duration of the activated state. Our study in rats, a most widely used species in neurobiology, shows that a distinct class of K^+ channels that can be activated by both free fatty acids and membrane tension exist in rat brain neurons. Arachidonic and other free fatty acids have been reported to modify the kinetic properties of ion channels either directly or indirectly (Rouzaire-Dubois, Gerard & Dubois, 1991; Behe, Sandmeier & Meves, 1992; Villarroel, 1993). Arachidonic acid or its metabolites has

been found to mediate the effect of certain neurotransmitters or hormones on ion channels (Buttner, Siegelbaum & Volterra, 1989; Premkumar *et al.* 1990; Schweitzer *et al.* 1990; Miller *et al.* 1992). Therefore, an agonist that causes formation of arachidonic acid would be expected to activate K^+ channels identified here. In our study, however, glutamate and dopamine, which have been reported to cause release of arachidonic acid in certain neurons, failed to activate the K^+ channels. Since we have not measured the release of arachidonic acid in our cultured cells we cannot easily determine whether the lack of activation was due to failure to generate sufficient arachidonic acid or due to unknown reasons. To determine the physiological role of the K^+ channels described here, future studies are planned to identify other potential agonists or stimuli that would cause their activation. The fatty acid-activated K^+ channels are likely to be important in pathological conditions such as brain ischaemia, epilepsy and stroke in which significant increases in cellular free arachidonic acid are known to occur. Molecules such as arachidonic acid (Williams, Errington, Lynch & Bliss, 1989), platelet-activating factor (Kato, Clark, Bazan & Zorumski, 1994) and nitric oxide (O'Dell, Hawkins, Kandel & Arancio, 1991) have been suggested to be involved in long term potentiation and in altering synaptic transmission in different parts of the brain. Such changes may involve the type of K^+ channels described in this study.

Behe, P., Sandmeier, K. & Meves, H. (1992). The effect of arachidonic acid on the M current of NG108-15 neuroblastomaxglioma hybrid cells. *Pflügers Archiv* **422**, 120–128.

Buttner, N., Siegelbaum, S. A. & Volterra, A. (1989). Direct modulation of *Aplysia* S-K^+ channels by a 12-lipoxygenase metabolite of arachidonic acid. *Nature* **342**, 553–555.

Calabresi, P., Maj, R., Pisani, A., Mercuri, N. B. & Bernardi, G. (1992). Long-term synaptic depression in the striatum: physiological and pharmacological characterization. *Journal of Neuroscience* **12**, 4224–4233.

Collier, T. J., Gallagher, M. J. & Sladek, C. D. (1993). Cryopreservation and storage of embryonic rat mesencephalic dopamine neurons for one year: comparison to fresh tissue in culture and neural grafts. *Brain Research* **623**, 249–256.

Collier, T. J., Sladek, C. D., Gallagher, M. J., Gereau, R. W. & Springer, J. E. (1990). Diffusible factors from adult rat sciatic nerve increase cell number and neurite outgrowth of cultured embryonic ventral mesencephalic tyrosine hydroxylase-positive neurons. *Journal of Neuroscience Research* **27**, 394–399.

Dumuis, A., Pin, J. P., Oomagari, K., Sebben, M. & Bockaert, J. (1990). Arachidonic acid released from striatal neurons by joint stimulation of ionotropic and metabotropic quisqualate receptors. *Nature* **347**, 182–184.

Fraser, D. D., Hoehn, K., Weiss, S. & MacVicar, B. A. (1993). Arachidonic acid inhibits sodium currents and synaptic transmission in cultured striatal neurons. *Neuron* **11**, 633–644.

Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, J. F. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv* **391**, 85–100.

Kandel, E. R. & O'Dell, T. J. (1992). Are adult learning mechanisms also used for development? *Science* **258**, 243–245.

Kato, K., Clark, G. D., Bazan, N. G. & Zorumski, C. F. (1994). Platelet-activating factor as a potential retrograde messenger in CA1 hippocampal long-term potentiation. *Nature* **367**, 175–179.

Keyser, D. O. & Alger, B. E. (1990). Arachidonic acid modulates hippocampal calcium current via protein kinase C and oxygen radicals. *Neuron* **5**, 545–553.

Kim, D. (1990). Regulation of K channels in cardiac myocytes by free fatty acids. *Circulation Research* **67**, 1040–1046.

Kim, D. (1992). A mechanosensitive K^+ channel in heart cells. Activation by arachidonic acid. *Journal of General Physiology* **100**, 1021–1040.

Kim, D. & Clapham, D. E. (1989). Potassium channels in cardiac cells activated by arachidonic acid and phospholipids. *Science* **244**, 1174–1176.

Kirber, M. T., Ordway, R. W., Clapp, L. H., Walsh, J. V. Jr & Singer, J. J. (1992). Both membrane stretch and fatty acids directly activate large conductance Ca^{2+}-activated K^+ channels in vascular smooth muscle cells. *FEBS Letters* **297**, 24–28.

Linden, D. J. & Routtenberg, A. (1989). *Cis*-fatty acids which activate protein kinase C, attenuate Na^+ and Ca^{2+} currents in mouse neuroblastoma cells. *Journal of Physiology* **419**, 95–119.

Madarasz, E., Kornyei, Z., Poulain, D. A. & Theodosis, D. T. (1992). Development of oxytocinergic neurons in monolayer cultures derived from embryonic, fetal and postnatal rat hypothalami. *Journal of Neuroendocrinology* **4**, 433–439.

Masuko, S., Nakajima, S. & Nakajima, Y. (1992). Dissociated high-purity dopaminergic neuron cultures from the substantia nigra and the ventral tegmental area of the postnatal rat. *Neuroscience* **49**, 347–364.

Meves, H. (1994). Modulation of ion channels by arachidonic acid. *Progress in Neurobiology* **43**, 175–186.

Miller, B., Sarantis, M., Traynelis, S. F. & Attwell, D. (1992). Potentiation of NMDA receptor current by arachidonic acid. *Nature* **355**, 722–725.

Nichols, C. G. & Lederer, W. J. (1991). Adenosine triphosphate-sensitive potassium channels in the cardiovascular system. *American Journal of Physiology* **261**, H1675–1686.

O'Dell, T. J., Hawkins, R. D., Kandel, E. R. & Arancio, O. (1991). Tests of the roles of two diffusible substances in long-term potentiation: Evidence for nitric oxide as a possible early retrograde messenger. *Proceedings of the National Academy of Sciences of the USA* **88**, 11285–11289.

Ordway, R. W., Singer, J. J. & Walsh, J. V. Jr (1991). Direct regulation of ion channels by fatty acids. *Trends in Neurosciences* **14**, 96–100.

Ordway, R. W., Walsh, J. V. Jr & Singer, J. J. (1989). Arachidonic acid and other fatty acids directly activate potassium channels in smooth muscle cells. *Science* **244**, 1176–1179.

Petrou, S., Ordway, R. W., Singer, J. J. & Walsh, J. V. Jr (1993). A putative fatty acid-binding domain of the NMDA receptor. *Trends in Biochemical Sciences* **18**, 41–42.

Piomelli, D., Pilon, C., Giros, B., Sokoloff, P., Martres, M.-P. & Schwatz, J.-C. (1991). Dopamine activation of the arachidonic acid cascade as a basis for D1/D2 receptor synergism. *Nature* **353**, 164–167.

PIOMELLI, D., VOLTERRA, A., DALE, N., SIEGELBAUM, S. A., KANDEL, E. R., SCHWARTZ, J. H. & BELARDETTI, F. (1987). Lipoxygenase metabolites of arachidonic acid as second messengers for presynaptic inhibition of *Aplysia* sensory cells. *Nature* **328**, 38–43.

PREMKUMAR, L. S., GAGE, P. W. & CHUNG, S. H. (1990). Coupled potassium channels induced by arachidonic acid in cultured neurons. *Proceedings of Royal Society* B **242**, 17–22.

ROUZAIRE-DUBOIS, B., GERARD, V. & DUBOIS, J.-M. (1991). Modification of K^+ channel properties induced by fatty acids in neuroblastoma cells. *Pflügers Archiv* **419**, 467–471.

SCHWEITZER, P., MADAMBA, S. & SIGGINS, G. R. (1990). Arachidonic acid metabolites as mediators of somatostatin-induced increase of neuronal M-current. *Nature* **346**, 464–467.

SIGWORTH, F. J. & SINE, S. M. (1987). Data transformations for improved display and fitting of single channel dwell time histograms. *Biophysical Journal* **52**, 1047–1054.

SLADEK, C. D. & GALLAGHER, M. J. (1993). The stimulation of vasopressin gene expression in cultured hypothalamic neurons by cyclic adenosine 3′,5′-monophosphate is reversible. *Endocrinology* **133**, 1320–1330.

SUKHAREV, S. I., MARTINAC, B., ARSHAVSKY, V. Y. & KUNG, C. (1993). Two types of mechanosensitive channels in the *Escherichia coli* cell envelope: solubilization and functional reconstitution. *Biophysical Journal* **65**, 177–183.

VANDORPE, D. H., SMALL, D. L., DABROWSKI, A. R. & MORRIS, C. E. (1994). FMRFamide and membrane stretch as activators of the *Aplysia* S-channel. *Biophysical Journal* **66**, 46–58.

VILLARROEL, A. (1993). Suppression of neuronal potassium A-current by arachidonic acid. *FEBS Letters* **335**, 184–188.

WALLERT, M. A., ACKERMAN, M. J., KIM, D. & CLAPHAM, D. E. (1991). Two novel cardiac atrial K^+ channels, IK.AA and IK.PC. *Journal of General Physiology* **98**, 921–939.

WILLIAMS, J. H., ERRINGTON, M. L., LYNCH, M. A. & BLISS, T. V. P. (1989). Arachidonic acid induces a long-term activity-dependent enhancement of synaptic transmission in the hippocampus. *Nature* **341**, 739–742.

YANG, X.-C. & SACHS, F. (1989). Block of stretch-activated ion channels in *Xenopus* oocytes by gadolinium and calcium ions. *Science* **243**, 1068–1071.

Acknowledgements

This study was supported in part by NIH grant HL-48052 during the tenure of American Heart Association Established Investigatorship (D. K.).

Received 21 March 1994; accepted 20 October 1994.