A Pertussis Toxin-Sensitive 8-Lipoxygenase Pathway Is Activated by a Nicotinic Acetylcholine Receptor in Aplysia Neurons

TAMARA L. TIEMAN,1 DOUGLAS J. STEEL,2 YELENA GOR,1 JACSUE KEHOE,5 JAMES H. SCHWARTZ,3 AND STEVEN J. FEINMARK1,4
1Department of Pharmacology, 2Department of Pathology, 3Center for Neurobiology and Behavior, and 4Center for Molecular Therapeutics, College of Physicians and Surgeons, Columbia University, New York, New York 10032; and 5Laboratoire de Neurobiologie, Ecole Normale Supérieure, 75005 Paris, France

Received 24 May 2000; accepted in final form 5 February 2001

Tieman, Tamara L., Douglas J. Steel, Yelena Gor, JacSue Kehoe, James H. Schwartz, and Steven J. Feinmark. A pertussis toxin-sensitive 8-lipoxygenase pathway is activated by a nicotinic acetylcholine receptor in Aplysia neurons. J Neurophysiol 85: 2150–2158, 2001. Acetylcholine (ACh) activates two types of chloride conductances in Aplysia neurons that can be distinguished by their kinetics and pharmacology. One is a rapidly desensitizing current that is blocked by α-conotoxin-ImI and the other is a sustained current that is insensitive to the toxin. These currents are differentially expressed in Aplysia neurons. We report here that neurons that respond to ACh with a sustained chloride conductance also generate 8-lipoxygenase metabolites. The sustained chloride conductance and the activation of 8-lipoxygenase have similar pharmacological profiles. Both are stimulated by suberyldicholine and nicotine, and both are inhibited by α-bungarotoxin. Like the sustained chloride conductance, the activation of 8-lipoxygenase is not blocked by α-conotoxin-ImI. In spite of the similarities between the metabolic and electrophysiological responses, the generation of 8-lipoxygenase metabolites does not appear to depend on the ion current since an influx of chloride ions is neither necessary nor sufficient for the formation of the lipid metabolites. In addition, the application of pertussis toxin blocked the ACh-activated release of arachidonic acid and the subsequent production of 8-lipoxygenase metabolites, yet the ACh-induced activation of the chloride conductance is not dependent on a G protein. Our results are consistent with the idea that the nicotinic ACh receptor that activates the sustained chloride conductance can, independent of the chloride ion influx, initiate lipid messenger synthesis.

INTRODUCTION

Application of neurotransmitters to Aplysia neurons activates specific lipoxygenase pathways. The application of histamine to identified neurons in the abdominal ganglion of Aplysia results in the release of arachidonic acid, through the action of a phospholipase A2 (Shapiro et al. 1988), and the conversion of arachidonic acid to bioactive 12-lipoxygenase products (Piromelli et al. 1988, 1989). Similarly, the application of acetylcholine (ACh) to Aplysia nervous tissue leads to the generation of 8-lipoxygenase metabolites. Previously we showed that ACh induces the generation of 8(R)-hydroperoxyeicosatetraenoic acid [8(R)-HPETE], which can be reduced both enzymatically and nonenzymatically to 8(R)-hydroxyeicosatetraenoic acid [8(R)-HETE]. In addition, 8-HPETE is enzymatically converted to a ketone, 8-ketoehicosatetraenoic acid (8-KETE) (Steel et al. 1997). Other more polar enzymatic products probably related to hepoxilin have been tentatively identified (Tieman and Feinmark, unpublished data). These metabolites comprise the lipids of the 8-lipoxygenase family.

8-Lipoxygenase is a member of a large family of enzymes with members distributed widely in both plants and animals (Brash 1999; Kuhn and Thiele 1999). Since a major function of the well-characterized lipoxygenases is to generate second messengers and signaling molecules, it seems likely that the ACh-induced metabolites of 8-lipoxygenase also play a specific role in Aplysia neural function.

To further our understanding of how 8-lipoxygenase is activated by ACh, we characterized the ACh receptor (AChR) that activates 8-lipoxygenase metabolism. ACh is known to activate four pharmacologically distinct receptors. One is a G-protein-linked, metabotropic receptor that activates a potassium conductance (Kehoe 1972a; Sasaki and Sato 1987). The other three are ionotropic AChRs, one that mediates a nonspecific cationic conductance (Ascher et al. 1978) with the other two being distinct chloride conductances (Kehoe and McIntosh 1998). One of the chloride-dependent responses is rapidly desensitizing; the other is sustained during agonist application.

Both of the AChRs that control chloride conductances are activated by nicotine and suberyldicholine and both are blocked by α-bungarotoxin (α-BTx); only the AChR that mediates the rapidly desensitizing chloride current is blocked by α-conotoxin-ImI (α-CTx-ImI), however (Kehoe and McIntosh 1998; Kehoe et al. 1976).

Here we provide pharmacological evidence that 8-lipoxygenase metabolism is activated by the nicotinic AChR that mediates the sustained chloride conductance. Several of the properties of the 8-lipoxygenase activation and the sustained chloride conductance are similar. Both are activated by suberyldicholine and nicotine, blocked by α-BTx, and unaffected by α-CTx-ImI. Because nicotinic receptors presumably are all ionotropic, the activation of 8-lipoxygenase metabolism by a nicotinic receptor is unexpected.
Methods

Biochemical experiments

*Aplysia californica* weighing 20–100 g were obtained from Marinus (Long Beach, CA) and the Miami *Aplysia* Facility (Miami, FL) and maintained in artificial sea water (ASW) at 15°C prior to dissection. Central ganglia were removed by dissection from animals anesthetized by injecting isotonic MgCl₂ (Schwartz and Swanson 1987). The ganglia were transferred to a small volume of ASW (Eisenstadt et al. 1973), chloride-free ASW [(in mM) 460 NaOH, 55 MgSO₄, 11 Ca(OH)₂, 10 KOH, 10 Tris base, and 546 methanesulfonic acid, pH adjusted to 7.6 with NaOH], or high Mg²⁺/low Ca²⁺ solution [(in mM) 230 NaCl, 10 KCl, 2 NaHCO₃, 22 MgCl₂, and 10 HEPES plus 50× MEM amino acids solution (4 ml/l; without L-glutamine; Gibco, Grand Island, NY), 100× MEM nonessential amino acids solution (2 ml/l; 10 mM), and 100× MEM vitamin solution (5 ml/l), the pH adjusted to 7.6 at room temperature]. Neural components (neurons and neuropil) were obtained by removing the connective tissue sheaths of ganglia from 60 to 100 g animals. Ganglia were pinned to a silicone plastic (Sylgard, Dow Corning, Midland, MI) and the connective tissue sheath removed under a dissecting microscope.

The following identified neurons were isolated from mature animals weighing 20–50 g (Camardo et al. 1983): cells B3, 6, 8, 9, and 10 and certain neighboring cells from the buccal ganglia (Gardner and Kandel 1977); cells of the medial group in the pleural ganglia (Kehoe 1972a); and cells of the RB group of the abdominal ganglion (Kandel et al. 1967). Pleural, buccal, and abdominal ganglia were incubated with protease (10 mg/ml; Sigma Type IX; Sigma Chemical, St. Louis, MO) in modified Leibovitz 15 (L15; Sigma) medium. To make this medium, additional salts [(in mM) 385 NaCl, 10 KCl, 28 MgcCl₂, 27 MgSO₄, 2.3 NaHCO₃, 35 glucose, and 11 CaCl₂] and penicillin-streptomycin (1% vol/vol; Gibco; 10,000 units of penicillin; Gibco, Grand Island, NY) were added to L15 medium, and the final solution adjusted to 35°C for 90–150 min depending on the age of the animals. The treated ganglia were washed three times for at least 5 min each in modified L15 medium before the connective tissue sheath was removed with fine forceps. Cells were isolated from ganglia with a glass needle and transferred to glass test tubes containing ASW.

Labeling and incubation of neural tissue

In most experiments, membrane lipids were labeled by incubating the neural components with [*H*]arachidonic acid (10 μCi/200 μl ASW; 100 μCi/ml; DuPont/NEN, Boston, MA) in microcentrifuge tubes for 2 h at 15°C. Neural components were washed by gently replacing the incubation fluid with ASW containing the test agonist. Isolated cells were labeled immediately after the dissection for 2 h in glass tubes containing [*H*]arachidonic acid in ASW (5 μCi/1 ml); in most experiments, these cells were washed to remove excess radioactive material, and then resuspended in ASW with [*H*]arachidonic acid for 2 h, washed twice with ASW (500 μl) containing bovine serum albumin (0.5% wt/vol), and then incubated for 6 h with the holotoxin (0.1 μg/ml) or toxin that had been heat inactivated (100°C for 5 min).

Extraction of lipids

Before we extracted the lipids, we added 8-HETE or 12-HETE to each sample as internal standard. The samples were then acidified to pH 3.5 with HCl and extracted with diethyl ether (Steel et al. 1997). The lipid extract was evaporated to dryness under reduced pressure and resuspended in mobile phase for chromatographic analysis or stored in ethanol.

Reverse phase-high performance liquid chromatography (RP-HPLC)

Lipid extracts were fractionated on a NovaPak C₁₈ column (Waters Chromatography, Milford, MA) eluted isocratically at 0.7 ml/min with acetonitrile/water (50:50, vol/vol; pH adjusted to 4.5 with acetic acid). Between injections, the column was washed with acetonitrile to elute nonpolar lipids. HPLC analyses were performed on a Hewlett-Packard 1090M (Hewlett-Packard Instruments, Paramus, NJ) with a photodiode array UV detector in series with a flow-through radioactivity monitor (B-Ram, IN/US Systems, Tampa, FL). The internal standard was quantified by its absorbance at 235 nm and used to correct for losses during extraction. Radioactivity was measured with Scintiflow software and normalized to the recovery of the internal standard. Samples were mixed with UniverSol scintillation fluid (ICN Biochemical, Costa Mesa, CA) in a ratio of 1:3.

Electrophysiological experiments

Electrophysiology was done on the neurons described in the preceding text for the isolated cell experiments. Ganglia were prepared as described by (Kehoe 1985). The ASW used in these studies contained (in mM) 480 NaCl, 10 KCl, 10 CaCl₂, 50 MgCl₂, and 10 Na-HEPES, pH 7.8. The composition of the chloride-free ASW was the same as given in the preceding text for the biochemical experiments. All drugs used were obtained from Sigma.

Recordings were usually made in the two-electrode voltage-clamp mode as described (Kehoe 1985). For the experiments designed to assess the role of a G protein in the chloride-dependent responses, whole cell patch-clamp methods were used (Hamill et al. 1981; Kehoe 1985). The ASW used in these studies contained (in mM) 411 K₂SO₄, 8.3 Na₂SO₄, 1 CaCl₂, 2 MgCl₂, and 5 EGTA (buffered with 20 mM K-HEPES to pH 7.4). For control recordings, GTP (1 mM) and ATP (10 mM) were included in the internal solution. In some experiments, GTP y-S (10 mM) or GDP-β-S (10 mM) was also included with or without ATP and GTP. Continuous recordings were made on a Servogor 340 paper recorder and on a digital audio tape recorder. Records of agonist-elicited currents were digitized and sampled on-line using a Cambridge Electronic Design 1401 interface and the whole cell electrophysiology program from Strathclyde Electrophysiological Software. Agonists and antagonists were applied using the fast perfusion system described by Kehoe and McIntosh (1998).

Statistics

Lipid products were quantified and normalized to an internal standard. Data are reported as the means ± SE. Differences between
RESULTS
As shown in the representative HPLC trace of Fig. 1, application of ACh to Aplysia neuronal tissue induced the formation of 8-HETE and 8-KETE. The effect of ACh was dose dependent: lipid metabolites were produced at concentrations of the transmitter as low as 1 mM and increased up to 1 mM, the highest dose tested. The response to ACh was specific. We tested histamine (previously shown to activate 12-lipoxygenase in Aplysia neurons (Piomelli et al. 1987a), 5-HT, GABA, glutamate, octopamine, dopamine, FMRFamide, and myomodulin. No neurotransmitter other than ACh led to a significant production of either 8-HETE or 8-KETE (Fig. 2). In addition, depolarization of neurons with KCl (60 mM) did not cause the generation of the metabolites. In two trials, the mean 8-HETE production was: ASW, 375 cpm; ACh (100 μM), 5,635 cpm; and KCl (60 mM), 690 cpm.

Pharmacology of 8-lipoxygenase activation
To identify the receptor that mediates activation of 8-lipoxygenase, we tested several cholinergic agonists and antagonists previously shown to be effective at Aplysia ACh receptors. Carbachol, a nonspecific cholinergic agonist, was as effective as ACh in activating this pathway (data not shown). Nicotine and suberyldicholine, which activate only the receptors that mediate chloride conductances (Ger and Zeimal 1977; Kehoe 1979; Kehoe and McIntosh 1998), also were effective (Fig. 3). Of the cholinergic antagonists tested, only α-BTX blocked the activation of 8-lipoxygenase (Fig. 4). The concentration of toxin used in this experiment has been shown to block the ACh-activated chloride conductances in Aplysia (Kehoe et al. 1976) but does not interfere with the known metabotropic AChR. Tubocurarine, like atropine, hexamethonium, and TEA failed to inhibit the response (Fig. 4). Although tubocurarine has been shown to be a weak antagonist of the two ACh-
activated chloride conductances (Kehoe and McIntosh 1998), concentrations higher than those used here would have been needed to block the sustained chloride-dependent response.

Two pharmacologically distinct receptors activate ACh-mediated increases in chloride conductance: one mediates a rapidly desensitizing chloride conductance that is blocked by $\alpha$-CTx-ImI; the other, a sustained chloride conductance that is not affected by the toxin (Kehoe and McIntosh 1998). We found that $\alpha$-CTx-ImI did not inhibit the production of 8-lipoxygenase metabolites induced by ACh in intact neural components (Fig. 5) nor did the toxin block suberyldicholine-activated 8-lipoxygenase metabolism either in intact neural components or in isolated cells (Table 1).

Arecoline, which activates a G-protein-linked receptor mediating a potassium conductance but does not activate a chloride conductance in Aplysia neurons (Kehoe 1972b), also stimulated the production of 8-lipoxygenase products (Fig. 6). Arecoline failed to activate 8-lipoxygenase metabolism when...
Neural components or isolated cells were incubated with suberyldicholine (100 μM) with or without α-conotoxin Iml (10 μM) as described in the text. 8-HETE was quantified by high performance liquid chromatography (HPLC) analysis. Results are presented as mean cpm ± SE from 4 experiments for neural components and 3 experiments for isolated cells. Suberyldicholine induced production of 8-HETE when compared to artificial seawater (ASW) alone (P < 0.05) either in the presence or absence of conotoxin (CTx). There was no significant difference between 8-HETE production induced by suberyldicholine or suberyldicholine together with CTx.

the experiments were repeated in high Mg2+/low Ca2+ seawater (220 mM Mg2+/1 mM Ca2+), a condition under which chemical synaptic transmission is blocked (Gardner 1977). In contrast, under the same conditions, ACh-induced activation of 8-lipoxygenase metabolism persisted (Fig. 6). Thus arecoline does not appear to activate the 8-lipoxygenase-linked AChR directly, but rather indirectly through a polysynaptic, ACh-dependent mechanism. This idea was supported by the finding that α-BTx blocks arecoline-induced 8-lipoxygenase activation (data not shown).

Co-localization of an ACh-induced chloride conductance with 8-lipoxygenase metabolism

The AChR that activates the α-CTx-Iml-insensitive, sustained chloride conductance is differentially distributed in *Aplysia* neurons (Kehoe and McIntosh 1998). If this receptor is also linked to 8-lipoxygenase metabolism, 8-lipoxygenase activity would be expected to be similarly distributed. We therefore used isolated identified cell bodies to see if the receptors that mediate the chloride-dependent responses occur in the same cells as the ACh-induced production of 8-lipoxygenase metabolites. We first tested selected cells from the buccal ganglia and medial cells from pleural ganglia in which ACh elicits both of the chloride-dependent responses (Fig. 7, A and B, insets). These neurons generated metabolites of the 8-lipoxygenase pathway when exposed to nicotine (Fig. 7, A and B). ACh elicits only an inward, cationic current in RB cells from the abdominal ganglia (Fig. 7C, inset, bottom trace). Neither suberyldicholine (see inset) nor nicotine (not shown) elicits a change in membrane conductance. Thus AChRs that mediate chloride conductances are absent (Fig. 7C, inset, top trace). Therefore as expected, exposure of RB cells to several cholinergic agonists, including nicotine and suberyldicholine (Fig. 7C), failed to elicit 8-HETE production.

Chloride influx and ACh-induced 8-lipoxygenase metabolism

GABA and glutamate both induce increases in chloride conductance in *Aplysia* neurons (King and Carpenter 1989). We examined the action of these two transmitters using identified neurons known to have the AChRs that mediate the chloride conductances. GABA (1 mM) and glutamate (200 μM) were applied to these cells at concentrations sufficient to activate a chloride conductance similar in amplitude to that elicited by ACh in the same cell types. Neither transmitter activated 8-lipoxygenase metabolism, suggesting that chloride influx alone is not sufficient for activating lipid metabolism. To determine whether the influx of chloride is necessary for the activation of 8-lipoxygenase, we replaced chloride with an impermeant anion, methanesulfonic acid, in a modified, chloride-free ASW. Under these conditions, ACh failed to elicit influx of chloride ion and the generation of 8-lipoxygenase products was unaffected (Fig. 8).

Activation of 8-lipoxygenase is blocked by pertussis toxin

ACh-activated chloride conductances do not depend on a G protein since they persist during whole cell recordings made in the absence of ATP and GTP in the pipette solution. Under the same conditions, the potassium conductance that is known to be G-protein dependent is eliminated (Kehoe 1994). Furthermore, chloride-dependent responses persist in cells in which G proteins are blocked by GDP-β-S or are “desensitized” by GTP-γ-S (data not shown). Is a G protein involved in ACh-induced 8-HETE synthesis? Shapiro et al. (1988) showed that agonist-induced release of arachidonic acid from *Aplysia* neuronal membrane lipids is mediated by G proteins and blocked by PTx. Suberyldicholine-activated production of 8-HETE was reduced when neural components are treated with PTx (Fig. 9A; 89% inhibition) but was not affected when inactivated PTx was used (data not shown). The release of free arachidonic acid in these experiments also was reduced when the neural components were treated with PTx (Fig. 9B; 65% inhibition), as would be expected if the activation of a phospholipase is blocked.

DISCUSSION

The known lipoxygenases generate metabolites from arachidonic acid that can act as signaling molecules, both between cells and as second messengers. Thus 5-lipoxygenase produces

![FIG. 6. Arecoline stimulates 8-lipoxygenase metabolism. *Aplysia* ganglia were dissected in normal ASW or in a high Mg2+/low Ca2+ seawater. Neural components were then labeled with [3H]arachidonic acid for 2 h as described in the legend to Fig. 1 and incubated with ACh (100 μM; ○) or arecoline (100 μM; ■) for 10 min. The lipids were extracted and analyzed as in the legend to Fig. 1. Arecoline is without effect in the high Mg2+/low Ca2+ seawater. A plausible explanation is that arecoline stimulates 8-lipoxygenase metabolism polysynaptically. Data are expressed as percent of control release (corrected for losses during extraction) and are mean values ± SE for 9 independent experiments. *P < 0.01; **P < 0.05.](http://jn.physiology.org/DownloadedFrom)
the leukotrienes that play an important role in neutrophil function during inflammation and in asthma (Samuelsson 1983), while 8-lipoxygenase generates molecules that regulate oocyte maturation in some invertebrates (Holland and East 1985; Meijer et al. 1986).

In the nervous system, the actions produced by lipoxygenase metabolites are cell- and neurotransmitter-specific. In neurons of Aplysia, arachidonic acid is metabolized by 12-lipoxygenase to produce substances that mimic the actions of the neurotransmitter that activated their production. For example, application of 12-lipoxygenase metabolites produces a dual-action response in cell L14 (rapid depolarization followed by a slow hyperpolarization), mimicking the responses evoked by the neurotransmitter, histamine (Piomelli et al. 1989), and a lipid from this pathway causes a hyperpolarization in sensory neurons like that induced by FMRFamide, which results in desensitization (Piomelli et al. 1987b). We now show that 8-lipoxygenase products are generated in specific Aplysia neurons in response to ACh, and we have characterized the receptor pharmacologically. While we have not yet identified their physiological roles, we presume that these metabolites also act within neurons as second messengers or as intercellular or retrograde signaling molecules.

There are four AChR known to exist in Aplysia neurons. One metabotropic receptor mediates a potassium conductance, and three ionotropic receptors mediate a nonspecific cation conductance and two distinct chloride conductances (one that is rapidly desensitizing and the other that is sustained).
arecoline-induced 8-HETE production is blocked by α-BTx, an antagonist that does not inhibit the metabotropic receptor known to be activated by arecoline. Rather the response to arecoline is the result of at least two AChRs linked polysynaptically, one a metabotropic arecoline receptor and the second the nicotinic AChR that mediates the sustained chloride response.

We found that activation of the sustained chloride conductance and activation of 8-lipoxygenase metabolism occur together in the same cells: both are observed in identified neurons from the buccal and pleural ganglia and both are absent from RB cells of the abdominal ganglia. This suggests that both the chloride conductance and the 8-lipoxygenase metabolism are activated by the same receptor. But are the chloride conductance and the lipid metabolism mediated by the same molecular entity? The pharmacological similarities between the biochemical and electrophysiological events triggered by ACh and their correlated expression in selected cells indicate that the two responses are mediated by the same AChR, even though the influx of chloride ion is neither necessary nor sufficient for activating 8-lipoxygenase.

A possible way in which an ionotropic receptor might trigger metabotropic activity is through a receptor-mediated increase in intracellular Ca$^{2+}$. Some nicotinic AChRs are highly permeable to Ca$^{2+}$ (McGehee and Role 1995), and Ca$^{2+}$-dependent chloride conductances are present in many neurons. Moreover, release of arachidonic acid from membrane phospholipids is usually a prerequisite for its metabolism and the release often depends on the activation of a Ca$^{2+}$-dependent phospholipase. Nevertheless, a role for Ca$^{2+}$ in the ACh-induced activation of 8-lipoxygenase studied here can be excluded since there is no evidence for an ACh-dependent Ca$^{2+}$ flux through the receptors that activate the sustained chloride conductance. First, there is no inward current in the selected cells used for these experiments, even when the agonists are applied by fast perfusion. Second, the rapid kinetics of the ACh-induced increase in chloride conductance strongly suggest that the change in conductance results from direct activation of a ligand-gated chloride channel. Finally, the ACh-induced chloride responses persist in external solutions that are Ca$^{2+}$-free, as well as in cells that have been loaded with bis-(o-aminophenoxy)-N,N,N′,N′-tetraacetic acid (BAPTA; 10 mM) (Kehoe, unpublished data).
The sustained chloride conductance is mediated by an ionotropic AChR, but the nicotinic AChR that activates 8-lipoxygenase appears to have metabotropic characteristics. Thus activation of 8-lipoxygenase depends on a PTx-sensitive G protein (Fig. 9) that presumably links the receptor to a phospholipase by a mechanism that is similar to the way that histamine causes arachidonic acid to be released in other identified Aplysia neurons (Shapiro et al. 1988; Vogel et al. 1989). In contrast, the ACh-induced chloride conductance does not depend on the activation of a G protein. Although no AChRs have yet been cloned from Aplysia, ionotropic and metabotropic receptors known to date have been found to be similar across phylogeny (Bertrand and Changeux 1995; Le Novere and Changeux 1999; Sargent 1993).

Ionotropic receptors are not usually linked to metabotropic activities by G proteins, but some exceptions have been reported. Both a postsynaptic neuronal AMPA receptor (Wang et al. 1997) and a presumptive presynaptic kainate receptor (Rodriguez-Moreno and Lerma 1998) appear to initiate metabotropic events through PTx-sensitive G proteins. In fact, Wang et al. (1997) immunoprecipitated the AMPA receptor together with a G protein, suggesting an unexpected physical interaction between this ionotropic receptor and a metabotropic effector molecule. It has further been shown that activation of the G protein is independent of the ion flux through the AMPA-activated channel. Further, it is interesting that the G-protein-linked kainate receptor activates a phospholipase C since the AChR that we have studied also must activate a phospholipase, although most likely a phospholipase A2. Rather than a direct interaction between the ionotropic receptor and the G protein, it is possible that the AChR initiates the synthesis or release of an as yet unknown factor that subsequently activates the G protein causing lipid metabolism through the 8-lipoxygenase pathway. This mechanism has recently been proposed for the nicotine-induced activation of MAP kinase in small-cell lung carcinoma cells (Cattaneo et al. 1997).

Another possible explanation for the paradoxical nature of a nicotinic AChR having metabotropic properties would be a physical interaction between two receptor types as has recently been proposed for the nicotine-induced activation of MAP kinase in small-cell lung carcinoma cells (Cattaneo et al. 1997).

This work was supported by National Institutes of Health Grants NS-29832 (S. J. Feinmark) and MH-00921 (J. H. Schwartz), by a Université Pierre et Marie Curie Visiting Professorship (J. H. Schwartz), and by Centre National de la Recherche Scientifique Grant UMR 8544 (J. Kehoe).

REFERENCES


