Br. J. Pharmacol. (1994), **111**, 469–472

Inhibition of olfactory cyclic nucleotide-activated current by calmodulin antagonists

Steven J. Kleene

Department of Anatomy and Cell Biology, University of Cincinnati, 231 Bethesda Avenue, ML 521, Cincinnati, OH 45267-0521, U.S.A.

1 In amphibian olfactory receptor neurones, much of the depolarizing current in response to odours is carried by cationic channels that are directly gated by cyclic AMP. The effects of four calmodulin antagonists on the cyclic AMP-activated receptor current were studied in single olfactory cilia of the frog.

2 Two antagonists, W-7 and trifluoperazine, were potent and reversible inhibitors of the cyclic AMP-activated current. IC_{50} values were 5 μM for W-7 and 13 μM for trifluoperazine. A third antagonist, calmidazolium, irreversibly blocked the current. The fourth, mastoparan, had little effect.

3 Calmodulin was unable to reverse the effects of W-7 and trifluoperazine, suggesting that these inhibitors act directly on the cyclic AMP-gated channels.

4 Neither W-7 nor trifluoperazine inhibited a Ca^{2+}-activated Cl^- current which also contributes to the odorant response. These compounds thus allow the two components of the olfactory receptor current to be discriminated.

Keywords: Olfaction; transduction; receptor neurone; cilia; cyclic nucleotide-gated channel; calmodulin antagonists; W-7; trifluoperazine

Introduction

In amphibians, an adenosine 3′:5′-cyclic monophosphate (cyclic AMP)-mediated cascade initiates olfactory transduction. This occurs in the cilia of the olfactory receptor neurones (Kurahashi, 1989; Firestein *et al.*, 1990; Lowe & Gold, 1991). Many odorants increase the activity of adenylate cyclase in the cilia (Pace *et al.*, 1985; Sklar *et al.*, 1986; Shirley *et al.*, 1986; Boekhoff *et al.*, 1990; Bruch & Teeter, 1990). The cyclic AMP formed directly gates channels in the ciliary membrane (Nakamura & Gold, 1987), leading to a depolarizing inward current carried by cations. The cilia contain a high density of cyclic AMP-gated channels (Lowe & Gold, 1993; Kurahashi & Kaneko, 1993), and it has been confirmed in intact neurones that these channels conduct much of the receptor current in response to odours (Kurahashi, 1990; Firestein *et al.*, 1991; Lowe & Gold, 1993). If external Ca^{2+} is present, Ca^{2+} enters through the cyclic AMP-gated channels, activates Cl^- channels, and gives rise to a secondary receptor current carried by Cl^- (Kurahashi & Yau, 1993; Kleene, 1993).

It is useful to have pharmacological agents that can distinguish between the cationic and Cl^- components of the olfactory receptor current. I now report that W-7 and trifluoperazine are potent and reversible inhibitors of the cationic current that flows through the cyclic AMP-gated channels. These compounds have almost no effect on the Ca^{2+}-activated Cl^- portion of the receptor current. W-7 and trifluoperazine are often used as calmodulin antagonists. In this case, however, evidence suggests that they act on the cyclic nucleotide-gated channels directly rather than through calmodulin.

Methods

Ciliary patch procedure

Single olfactory receptor neurones were isolated from the dorsal olfactory epithelium of the northern grass frog (*Rana pipiens*). A recording micropipette was brought near one of the cilia and suction applied until the end of the cilium entered the pipette. Suction was continued until the olfactory vesicle touched the tip of the pipette and a high-resistance seal formed. Then the pipette was raised briefly into the air, causing excision of the cilium from the cell. The cilium remained sealed inside the recording micropipette with the cytoplasmic face of the membrane exposed to the bath. The pipette containing the cilium could be quickly transferred through the air to various pseudointracellular baths without rupturing the seal. Additional details have been presented elsewhere (Kleene & Gesteland, 1991a).

Solutions

Extracellular solutions were used to bathe intact cells and to fill the recording pipettes. The cell suspension was stored in standard extracellular solution, which contained (mM): NaCl 115, KCl 3, HEPES 5, $MgCl_2$ 2, $CaCl_2$ 1 and NaOH 2 (pH 7.2). Recording pipettes contained extracellular solution made without $MgCl_2$ and $CaCl_2$. This omission prevented open-channel block of the cyclic AMP-gated channels by external Ca^{2+} and Mg^{2+} (Nakamura & Gold, 1987; Dhallan *et al.*, 1990; Zufall & Firestein, 1993). In addition, it prevented the secondary Cl^- current that depends on external Ca^{2+} (Kurahashi & Yau, 1993; Kleene, 1993). Patch formation was accomplished in standard extracellular solution, since it was nearly impossible to form high-resistance seals without divalent cations in the bath. The small amount of Ca^{2+} and Mg^{2+} sucked into the pipette during the patch procedure quickly diffused back into the pipette. After seal formation, this was detectable as a gradual increase in cyclic AMP-activated current at negative potentials, as the blocking effect of the external divalent cations was relieved. This process was complete within 1–3 min.

After a cilium was excised from a neurone, the pipette containing the cilium was transferred through a series of pseudointracellular solutions which bathed the cytoplasmic face of the ciliary membrane. The standard pseudointracellular solution contained (mM): KCl 110, NaCl 5, HEPES 5, $CaCl_2$ 0.8, BAPTA 2 and KOH 9 (pH 7.2). Free Ca^{2+} was determined to be 0.1 μM as described elsewhere (Kleene &

Gesteland, 1991b). When calmodulin was used, 1 μM calmodulin was added to a solution containing 0.9 μM free Ca^{2+}. Inhibitors were added to the pseudointracellular bath as indicated.

Electrical recording

Both the recording pipette and chamber were coupled to a List L/M-EPC7 patch-clamp amplifier by Ag/AgCl electrodes, each bathed in extracellular solution. All recordings were done under voltage clamp at room temperature (25°C). Current was adjusted to zero with the open pipette in the well in which the patching procedure was done. Both the bath and the pipette contained extracellular solution at this stage. After excision of a cilium, the pipette was transferred through a series of pseudointracellular baths. Each bath was connected by a salt bridge to a common reference bath. The salt bridge contained extracellular solution plus 5% (w/v) agarose (Sigma Type I). A correction was applied for the liquid junction potential between each pseudointracellular bath and its salt bridge (Hagiwara & Ohmori, 1982).

Voltage ramps (−100 to +100 mV, 0.2 mV ms^{-1}) were generated by pCLAMP software (Axon Instruments, Foster City, CA, U.S.A.). Current-voltage (*I-V*) records were acquired at a sampling rate of 500 Hz. In all records, an upward deflection represents increasing positive current from the bath into the pipette. Potentials are reported as bath (cytoplasmic) potential relative to pipette potential. Results of repeated experiments are reported as mean ± s.e.mean.

Materials

W-7 (N-(6-aminohexyl)-5-chloro-1-naphthalenesulphonamide) hydrochloride, trifluoperazine dihydrochloride, and calmidazolium (compound R24571) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), as were all other reagents. W-7 and calmidazolium were diluted from 10 mM stock solutions in ethanol. Dilutions of these inhibitors were sonicated to ensure solution. Mastoparan was synthetic (Sigma catalogue No. M5280), and calmodulin was from bovine brain (Sigma No. P2277).

Results

Recordings were made from single cilia excised from frog olfactory receptor neurones (Kleene & Gesteland, 1991a). One cilium of a cell was sucked inside a patch pipette. A high-resistance seal was made between the base of a cilium and the tip of the pipette, and the pipette containing the cilium was detached from the cell. The cilium remained inside the pipette with the cytoplasmic face of its membrane exposed to the bath (Kleene & Gesteland, 1991a).

Addition of 100 μM cytoplasmic cyclic AMP activated a large ciliary membrane conductance (Figure 1, top, curve '0'). This was due to cationic channels directly gated by cyclic AMP (Nakamura & Gold, 1987). Addition of a calmodulin antagonist, W-7, to the cytoplasmic bath caused a decrease in conductance that depended on the concentration of W-7 (Figure 1) and became stable within 1 min. Inhibition was nearly complete with 100 μM W-7 and was largely reversible. Within 3–5 min after removal of 100 μM W-7, 80 ± 5% (range 70 to 92%, $n = 4$) of the cyclic AMP-activated current returned. Another calmodulin antagonist, trifluoperazine, also produced nearly complete inhibition at 100 μM, although the IC_{50} was higher than that of W-7 (Figure 1). Inhibition took 1–2 min and was mostly reversible. Within 6–8 min after removal of 100 μM trifluoperazine, 83 ± 5% (range 65–103%, $n = 6$) of the cyclic AMP-activated current returned. Both W-7 and trifluoperazine were somewhat more effective at positive potentials, as reported for other inhibitors of cyclic nucleotide-gated channels (Frings *et al.*, 1992; Haynes, 1992; Kleene, 1993).

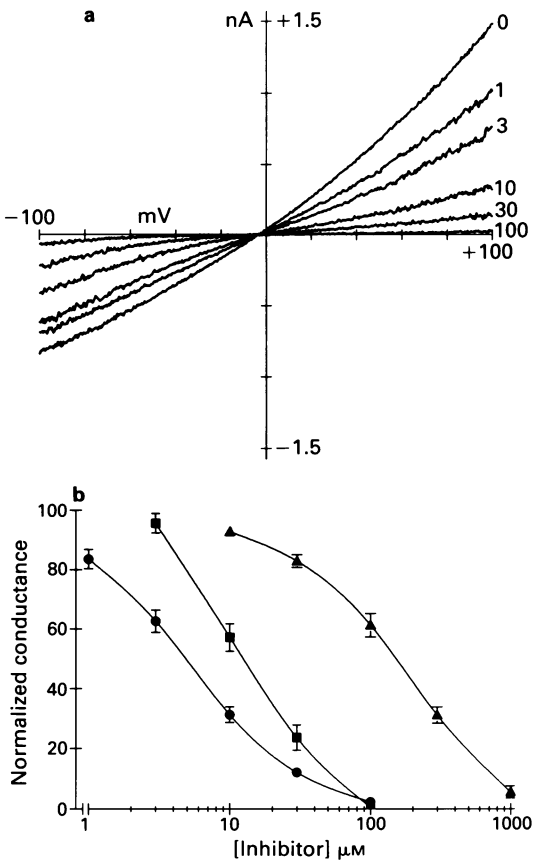

Figure 1 Inhibitors of the cyclic AMP-activated ciliary current. (a) A pipette containing a cilium was moved through baths containing a pseudointracellular solution plus 100 μM cyclic AMP and W-7 as shown (in μM). In each bath, the *I-V* relation was measured. The *I-V* curve in the absence of cyclic AMP and inhibitor (not shown) was subtracted from each record so that only the cyclic AMP-activated current is plotted. (b) The slope of the *I-V* plot, measured between −50 and +50 mV, is plotted against the concentration of inhibitor added. Inhibitors are W-7 (●), trifluoperazine (■), and amiloride (▲). All points are means of determinations in 7–9 cilia. For each experiment, conductance in the absence of inhibitor was defined as 100 and the other values were normalized to this. The maximum cyclic AMP-activated conductance (slope of the '0' curve between −50 and +50 mV) averaged 8.7 ± 4.3 nS ($n = 9$, range 4.3 to 15.0 nS) for the W-7 experiments, 8.1 ± 0.9 nS ($n = 8$, range 4.7 to 12.3 nS) for the trifluoperazine experiments, and 11.6 ± 1.2 nS ($n = 8$, range 8.1 to 17.9 nS) for the amiloride experiments. Where error bars are not shown, the s.e.mean was less than 3. Interpolation from the dose-response curves shown gave estimated IC_{50} values of 5 μM for W-7, 13 μM for trifluoperazine, and 150 μM for amiloride.

A third calmodulin antagonist, calmidazolium, caused a very slow and irreversible inhibition of the cyclic AMP-activated conductance. In the five cilia tested, effects of calmidazolium were first seen at 3–10 μM. Inhibition progressed for periods exceeding 10 min and continued even after transfer to a bath lacking the inhibitor. One calmodulin antagonist, the peptide mastoparan, had little effect on the ciliary cyclic AMP-activated conductance. At 10 μM, mastoparan inhibited the conductance by just 9 ± 2% (range 4 to 13%, $n = 5$). Inhibition by amiloride, which is not considered a calmodulin antagonist, is shown in Figure 1 for comparison. Amiloride has been shown to inhibit both the odour-induced receptor current (Persaud *et al.*, 1987; Frings & Lindemann, 1988) and the cyclic AMP-gated channels (Suzuki, 1990; Frings *et al.*, 1992).

Calmodulin was unable to reverse inhibition of the cyclic AMP-activated current by W-7 or trifluoperazine. In the presence of 5 μM W-7, this current was reduced by about

Table 1 Inhibitors of the cyclic nucleotide-activated current in olfactory receptor neurones

Inhibitor	IC_{50} (μM)	*Species*	*Reference*
Amiloride	–	Bullfrog	Suzuki, 1990
	17	Frog	Frings *et al.*, 1992
	70	Rat	Frings *et al.*, 1992
	150	Frog	This paper
Calmidazolium	–	Frog	This paper
D600	12	Frog	Frings *et al.*, 1992
3′,4′-Dichlorobenzamil (DCB, DCPA)	3.7	Frog	Kolesnikov *et al.*, 1990
	15	Frog	Kleene, 1993
	~4	Carp	Kolesnikov & Kosolapov, 1993
(+)-*cis*-Diltiazem	120	Frog	Frings *et al.*, 1992
(−)-*cis*-Diltiazem	47	Frog	Kolesnikov *et al.*, 1990
	70	Frog	Frings *et al.*, 1992
	128	Frog	Kleene, 1993
Nifedipine	–	Tiger salamander	Zufall & Firestein, 1993
Trifluoperazine	13	Frog	This paper
W-7	5	Frog	This paper

In all cases the inhibitor was applied to the cytoplasmic face of the membrane and little free Ca^{2+} or Mg^{2+} was present. Saturating concentrations of cyclic AMP were used in all but two cases (Suzuki, 1990; Kolesnikov & Kosolapov, 1993). In three cases (Suzuki, 1990; Zufall & Firestein, 1993; Kolesnikov & Kosolapov, 1993) single cyclic AMP-gated channels were studied. In the others, a macroscopic cyclic AMP-activated current was measured. Intact single cilia were studied in this paper and in Kleene (1993). The other studies used membrane patches excised from the soma or dendrite. A channel cloned from rat olfactory cDNA was inhibited by (−)-*cis*-diltiazem with an IC_{50} of 0.5–1 mM (Dhallan *et al.*, 1990). IC_{50} values were determined at different voltages as described in the individual references.

50% (Figure 1). Addition of 1 μM cytoplasmic calmodulin in the continued presence of the inhibitor had no significant effect ($n = 4$). In the presence of 10 μM trifluoperazine, 1 μM calmodulin caused a small additional decrease in the cyclic AMP-activated current ($n = 4$).

In amphibians, the olfactory receptor current includes both the cyclic AMP-activated cationic current and a Ca^{2+}-activated Cl^- current (Kurahashi & Yau, 1993; Kleene, 1993). It is thus useful to know whether the calmodulin antagonists allow the two currents to be distinguished. Neither W-7 or trifluoperazine substantially inhibited the ciliary Cl^- current activated by 300 μM cytoplasmic free Ca^{2+}. W-7 (100 μM) reduced this current by 5 ± 4% (range −11 to 19%, $n = 9$) and trifluoperazine (100 μM) by 5 ± 2% (range −1 to 10%, $n = 5$). Amiloride also reduced the Cl^- current by just 5 ± 3% (range −4 to 15%, $n = 5$).

Discussion

Two calmodulin antagonists, W-7 and trifluoperazine, have been found to be potent and reversible inhibitors of the cyclic AMP-activated cationic current that underlies olfactory transduction in amphibians. It seems likely that the compounds inhibit the cyclic AMP-gated channels directly rather than via calmodulin. Calmodulin was unable to reverse any of the partial inhibition caused by moderate doses of W-7 or trifluoperazine. Ca^{2+}-calmodulin does reduce the ligand affinity of the cyclic nucleotide-gated channels from rod photoreceptors (Hsu & Molday, 1993) and olfactory receptor neurones (Chen & Yau, 1993). However, this should only slightly reduce the current at the saturating concentrations of cyclic AMP used in this study.

W-7 and trifluoperazine must not be regarded as specific inhibitors. W-7 has been reported to block various Ca^{2+} channels and currents (Greenberg *et al.*, 1987; Ehrlich *et al.*, 1988; Nakazawa *et al.*, 1993), voltage-dependent Na^+ current (Ichikawa *et al.*, 1991), and Ca^{2+}-activated K^+ channels (Kihira *et al.*, 1990). Trifluoperazine inhibits Ca^{2+} currents (Greenberg *et al.*, 1987; Nakazawa *et al.*, 1993), voltage-dependent Na^+ current (Ichikawa *et al.*, 1991), Ca^{2+}-activated (McCann & Welsh, 1987; Kihira *et al.*, 1990; Ikemoto *et al.*, 1992) and ATP-sensitive (Müller *et al.*, 1991) K^+ channels, and GABA-activated Cl^- current (Yang & Zorumski, 1989). In ventricular and vascular myocytes, trifluoperazine depressed I_{Ca}, I_{Na}, and I_K (Klöckner & Isenberg, 1987). Many of these studies (Greenberg *et al.*, 1987; Klöckner & Isenberg, 1987; McCann & Welsh, 1987; Ehrlich *et al.*, 1988; Kihira *et al.*, 1990; Nakazawa *et al.*, 1993) included evidence suggesting that inhibition of channel activity was not mediated by calmodulin.

A number of compounds inhibit current through the olfactory cyclic nucleotide-gated channel (Table 1), but none can be considered specific inhibitors. W-7 and trifluoperazine inhibit at lower concentrations than most and are readily available from commercial suppliers. They allow discrimination between the two components of the olfactory receptor current present in amphibians. W-7 and trifluoperazine inhibit the cyclic nucleotide-activated cationic current but not the Ca^{2+}-activated Cl^- current. The same is true of DCB and (−)-*cis*-diltiazem (Kleene, 1993) and also amiloride. W-7 (Ichikawa *et al.*, 1991) and to a lesser extent trifluoperazine (Ikemoto *et al.*, 1992) are membrane-permeant, so they should be effective from either side of the neuronal membrane. For these reasons, W-7 and trifluoperazine are useful inhibitors of the cyclic nucleotide-activated channels that initiate olfactory transduction.

I am grateful to Robert Gesteland for critical reviews of the manuscript and to Barbara Cincush for technical assistance. This work was supported by National Institutes of Health grants R01 DC00926, P01 DC00347, and R01 DC00352.

References

BOEKHOFF, I., TAREILUS, E., STROTMANN, J. & BREER, H. (1990). Rapid activation of alternative second messenger pathways in olfactory cilia from rats by different odorants. *EMBO J.*, **9**, 2453–2458.

BRUCH, R.C. & TEETER, J.H. (1990). Cyclic AMP links amino acid chemoreceptors to ion channels in olfactory cilia. *Chem. Senses*, **15**, 419–430.

CHEN, T.-Y. & YAU, K.-W. (1993). Modulation by Ca^{2+}-calmodulin of cyclic nucleotide-gated cation channel of rat olfactory receptor neurons. *Soc. Neurosci. Abstr.*, **19,** 13.

DHALLAN, R.S., YAU, K.-W., SCHRADER, K.A. & REED, R.R. (1990). Primary structure and functional expression of a cyclic nucleotide-activated channel from olfactory neurons. *Nature*, **347,** 184–187.

EHRLICH, B.E., JACOBSON, A.R., HINRICHSEN, R., SAYRE, L.M. & FORTE, M.A. (1988). *Paramecium* calcium channels are blocked by a family of calmodulin antagonists. *Proc. Natl. Acad. Sci. U.S.A.*, **85,** 5718–5722.

FIRESTEIN, S., SHEPHERD, G.M. & WERBLIN, F.S. (1990). Time course of the membrane current underlying sensory transduction in salamander olfactory receptor neurons. *J. Physiol.*, **430,** 135–158.

FIRESTEIN, S., ZUFALL, F. & SHEPHERD, G.M. (1991). Single odor-sensitive channels in olfactory receptor neurons are also gated by cyclic nucleotides. *J. Neurosci.*, **11,** 3565–3572.

FRINGS, S. & LINDEMANN, B. (1988). Odorant response of isolated olfactory receptor cells is blocked by amiloride. *J. Membr. Biol.*, **105,** 233–243.

FRINGS, S., LYNCH, J.W. & LINDEMANN, B. (1992). Properties of the cyclic nucleotide-gated channels mediating olfactory transduction. *J. Gen. Physiol.*, **100,** 45–67.

GREENBERG, D.A., CARPENTER, C.L. & MESSING, R.O. (1987). Interaction of calmodulin inhibitors and protein kinase C inhibitors with voltage-dependent calcium channels. *Brain Res.*, **404,** 401–404.

HAGIWARA, S. & OHMORI, H. (1982). Studies of calcium channels in rat clonal pituitary cells with patch electrode voltage clamp. *J. Physiol.*, **331,** 231–252.

HAYNES, L.W. (1992). Block of the cyclic GMP-gated channel of vertebrate rod and cone photoreceptors by *l-cis*-diltiazem. *J. Gen. Physiol.*, **100,** 783–801.

HSU, Y.-T. & MOLDAY, R.S. (1993). Modulation of the cGMP-gated channel of rod photoreceptor cells by calmodulin. *Nature*, **361,** 76–79.

ICHIKAWA, M., URAYAMA, M. & MATSUMOTO, G. (1991). Anticalmodulin drugs block the sodium gating current of squid giant axons. *J. Membr. Biol.*, **120,** 211–222.

IKEMOTO, Y., YOSHIDA, A. & ODA, M. (1992). Blockade by trifluoperazine of a Ca^{2+}-activated K^+ channel in rat hippocampal pyramidal neurons. *Eur. J. Pharmacol.*, **216,** 191–198.

KIHIRA, M., MATSUZAWA, K., TOKUNO, H. & TOMITA, T. (1990). Effects of calmodulin antagonists on calcium-activated potassium channels in pregnant rat myometrium. *Br. J. Pharmacol.*, **100,** 353–359.

KLEENE, S.J. (1993). Origin of the chloride current in olfactory transduction. *Neuron*, **11,** 123–132.

KLEENE, S.J. & GESTELAND, R.C. (1991a). Transmembrane currents in frog olfactory cilia. *J. Membr. Biol.*, **120,** 75–81.

KLEENE, S.J. & GESTELAND, R.C. (1991b). Calcium-activated chloride conductance in frog olfactory cilia. *J. Neurosci.*, **11,** 3624–3629.

KLÖCKNER, U. & ISENBERG, G. (1987). Calmodulin antagonists depress calcium and potassium currents in ventricular and vascular myocytes. *Am. J. Physiol.*, **253,** H1601–H1611.

KOLESNIKOV, S.S. & KOSOLAPOV, A.V. (1993). Cyclic nucleotide-activated channels in carp olfactory receptor cells. *Biochim. Biophys. Acta*, **1150,** 63–72.

KOLESNIKOV, S.S., ZHAINAZAROV, A.B. & KOSOLAPOV, A.V. (1990). Cyclic nucleotide-activated channels in the frog olfactory receptor plasma membrane. *FEBS Lett.*, **266,** 96–98.

KURAHASHI, T. (1989). Activation by odorants of cation-selective conductance in the olfactory receptor cell isolated from the newt. *J. Physiol.*, **419,** 177–192.

KURAHASHI, T. (1990). The response induced by intracellular cyclic AMP in isolated olfactory receptor cells of the newt. *J. Physiol.*, **430,** 355–371.

KURAHASHI, T. & KANEKO, A. (1993). Gating properties of the cAMP-gated channel in toad olfactory receptor cells. *J. Physiol.*, **466,** 287–302.

KURAHASHI, T. & YAU, K.-W. (1993). Co-existence of cationic and chloride components in odorant-induced current of vertebrate olfactory receptor cells. *Nature*, **363,** 71–74.

LOWE, G. & GOLD, G.H. (1991). The spatial distributions of odorant sensitivity and odorant-induced currents in salamander olfactory receptor cells. *J. Physiol.*, **442,** 147–168.

LOWE, G. & GOLD, G.H. (1993). Contribution of the ciliary cyclic nucleotide-gated conductance to olfactory transduction in the salamander. *J. Physiol.*, **462,** 175–196.

MCCANN, J.D. & WELSH, M.J. (1987). Neuroleptics antagonize a calcium-activated potassium channel in airway smooth muscle. *J. Gen. Physiol.*, **89,** 339–352.

MÜLLER, M., DEWEILLE, J.R. & LAZDUNSKI, M. (1991). Chlorpromazine and related phenothiazines inhibit the ATP-sensitive K^+ channel. *Eur. J. Pharmacol.*, **198,** 101–104.

NAKAMURA, T. & GOLD, G.H. (1987). A cyclic nucleotide-gated conductance in olfactory receptor cilia. *Nature*, **325,** 442–444.

NAKAZAWA, K., HIGO, K., ABE, K., TANAKA, Y., SAITO, H. & MATSUKI, N. (1993). Blockade by calmodulin inhibitors of Ca^{2+} channels in smooth muscle from rat vas deferens. *Br. J. Pharmacol.*, **109,** 137–141.

PACE, U., HANSKI, E., SALOMON, Y. & LANCET, D. (1985). Odorant-sensitive adenylate cyclase may mediate olfactory reception. *Nature*, **316,** 255–258.

PERSAUD, K.C., DESIMONE, J.A., GETCHELL, M.L., HECK, G.L. & GETCHELL, T.V. (1987). Ion transport across the frog olfactory mucosa: the basal and odorant-stimulated states. *Biochim. Biophys. Acta*, **902,** 65–79.

SHIRLEY, S.G., ROBINSON, C.J., DICKINSON, K., AUJLA, R. & DODD, G.H. (1986). Olfactory adenylate cyclase of the rat. *Biochem. J.*, **240,** 605–607.

SKLAR, P.B., ANHOLT, R.R.H. & SNYDER, S.H. (1986). The odorant-sensitive adenylate cyclase of olfactory receptor cells. *J. Biol. Chem.*, **261,** 15538–15543.

SUZUKI, N. (1990). Single cyclic nucleotide-activated ion channel activity in olfactory receptor cell soma membrane. *Neurosci. Res.*, Suppl. **12,** S113–S126.

YANG, J. & ZORUMSKI, C.F. (1989). Trifluoperazine blocks GABA-gated chloride currents in cultured chick spinal cord neurons. *J. Neurophysiol.*, **61,** 363–373.

ZUFALL, F. & FIRESTEIN, S. (1993). Divalent cations block the cyclic nucleotide-gated channel of olfactory receptor neurons. *J. Neurophysiol.*, **69,** 1758–1768.

(Received September 13, 1993
Accepted October 13, 1993)