On the histamine-induced depolarization of the isolated superior cervical ganglion of the rat

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1 Using a grease-gap technique, we studied the action of histamine on the d.c. potential recorded between the internal carotid nerve and the main body of the isolated superior cervical ganglion of the rat.

2 A small, slow depolarization was evoked by 10–300 μM histamine. This response was not reduced by lowering the calcium concentration in the superfusing medium (from 2.5 to 0.1 mM), or by superfusing tetrodotoxin, N-methylatropine, or propranolol (all at 1 μM).

3 Mepyramine (10 nM) antagonized this depolarization, but cimetidine (10 μM), metiamide (30 μM), burimamide (10 μM) and impromidine (1 μM) did not. Two other agonists also evoked a mepyramine-sensitive slow depolarization. The rank order of potencies was histamine > N^α-methyl-histamine > 2-methyl-histamine.

4 At concentrations greater than 1 mM, histamine also evoked a larger, faster depolarization. This response was undiminished by reducing the calcium concentration of the medium to 0.1 mM or by adding 1 μM tetrodotoxin. The rank order of potency for the agonists was N^α-methyl-histamine > histamine $\approx$ 2-methyl-histamine. The histamine-induced fast response was not antagonized by any of the above-mentioned antagonists. It was slightly reduced by (+)-tubocurarine (100 μM) and N-methylbicyculline (100 μM) but such effects were not consistent with the blockade of nicotinic or GABA_A receptor-mediated responses.

5 It was concluded that histamine depolarized the isolated superior cervical ganglion of the rat by activating H₁ receptors. Relatively high concentrations of histamine also evoked a fast depolarization of this preparation, but this did not appear to be mediated by H₁, H₂ or H₃ receptors.

Keywords: Histamine; sympathetic ganglion; rat superior cervical ganglion; depolarization

Introduction

It is now well established that there is more than one type of receptor for histamine. In 1966, Ash & Schild defined the H₁ receptor as being sensitive to low concentrations of anti-histamine drugs (the pA₂ for mepyramine was >9). In 1972, Black *et al.* characterized the H₂ receptor using a novel selective antagonist, burimamide ($K_b = 7 \times 10^{-6}$ M). Subsequently H₁ receptors were shown to be linked to inositol phospholipid turnover (Subramanian *et al.*, 1980; Daum *et al.*, 1983) and H₂ receptors were reported to mediate an inhibition of adenylyl cyclase (Hegstrand *et al.*, 1976). The finding that histamine could inhibit its own release from brain slices (Arrang *et al.*, 1983) prompted the suggestion of another receptor. This H₃ receptor was clearly demonstrated by use of the selective agonist (R)- α -methyl-histamine and the selective antagonist thioperamide (Arrang *et al.*, 1987). At the same time, the H₃ receptor was demonstrated to mediate the histamine-induced inhibition of transmitter release in the guinea-pig mesenteric artery (Ishikawa & Sperelakis, 1987). In this preparation the H₂ antagonists cimetidine and metiamide were weak antagonists ($K_b > 30 \mu\text{M}$), whereas impromidine and burimamide had relatively high antagonist affinities (K_b s of 48 and 330 nM, respectively). The antagonist action of impromidine was intriguing since it was initially reported to be a potent H₂ receptor partial agonist (Ganellin, 1982).

The first neuronal actions of histamine to be described were a direct stimulant action on the feline superior cervical ganglion *in situ* and a facilitation of transmission across its synapse (Trendelenburg, 1954; 1957). The former action was shown to be sensitive to mepyramine before the H₁ receptor was defined (Trendelenburg, 1954). A facilitation of transmission through

the rabbit superior cervical ganglion was shown to be mediated by H₁ receptors but a depression of transmission in the same preparation was blocked by an H₂ antagonist (Brimble & Wallis, 1973). The activation of H₁ receptors on sympathetic neurones, and the subsequent release of noradrenaline, is thought to mediate the pressor action of intravenous histamine in the guinea-pig (Krstic, 1988). Recent intracellular studies in the guinea-pig superior cervical ganglion have revealed that the H₁ receptor mediates a depolarization associated with an increase in the neuronal input resistance (Christian *et al.*, 1989). On the rat superior cervical ganglion, however, histamine-induced depolarizations have been reported to be small or absent (Watson, 1970), but, as seen on the rabbit ganglion, an H₁-mediated facilitation and an H₂-mediated depression of transmission have been observed (Snow & Weinreich, 1987).

We observed that histamine does induce a small, slow depolarization of rat superior cervical ganglion cells *in vitro*. This paper describes our investigation of the pharmacology of this slow response which was compared with that of a fast depolarization induced by relatively high concentrations of histamine.

Methods

Male Sprague-Dawley rats (150–200 g) were killed by stunning followed by exsanguination. Two superior cervical ganglia were excised from each animal. Each ganglion was desheathed and set up in a three compartment bath as previously described (Newberry & Priestley, 1987). The d.c. potential between the ganglion body and the internal carotid nerve was recorded across a grease-gap with Ag/AgCl electrodes. The central compartment in which ganglion body was immersed (volume ca. 0.5 ml) was perfused with an aqueous medium (25°C) at ca. 2 ml min⁻¹. This medium contained (in mM):

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NaCl 125, NaHCO₃ 25, D-glucose 10, CaCl₂ 2.5, KCl 2, KH₂PO₄ 1 and MgSO₄ 1, and was equilibrated with 5% CO₂ in O₂.

Histamine (100 μ M) was applied to the ganglion body via the perfusion system for 1 min every 45 min until two equal responses were obtained. After the recovery of the last of these responses, the ganglion was superfused with an antagonist for one hour or, as a control, the ganglion was superfused with unadulterated medium for the same length of time. The concentration-response relationship of an agonist was then determined by 1 min superfusions at intervals of 45–60 min, in an ascending order of concentration. The peak responses of the agonists were expressed as ratios. They were related to the depolarization evoked by the last application of histamine prior to the 1 h incubation. This protocol was rigidly adhered to for the reasons given in the Results. The relative response of a given concentration of agonist on a number of ganglia was cumulated as the geometric mean ($\pm$ s.e.mean).

To confirm the antagonist properties of a given compound on GABA_A and nicotinic responses, we determined its IC₅₀ against 10 μ M GABA and 30 μ M 1,1-dimethyl-4-phenylpiperazinium (DMPP), respectively. The IC₅₀ was the concentration necessary to reduce the approximately half-maximal agonist response by 50%.

Burimamide, metiamide, impromidine trihydrochloride, N²-methyl-histamine dihydrochloride and 2-methyl-histamine dihydrochloride were kind gifts from Smith, Kline and French. Histamine dihydrochloride, mepyramine (pyrilamine) maleate, cimetidine, (+)-tubocurarine chloride, N-methylatropine nitrate, propranolol, γ -aminobutyric acid, 1,1-dimethyl-4-phenylpiperazinium iodide and N-methyl-bicuculline chloride were obtained from Sigma Chemical Company. The last was also supplied by Research Biochemicals Inc., as was mecamlamine. Tetrodotoxin was purchased from Sankyo.

An *n* number refers to the number of ganglia, each from a different rat, which were tested under a particular condition.

Results

Histamine (10–300 μ M) routinely induced a small, slow depolarization of the rat superior cervical ganglion. To obtain the largest response to a given concentration of histamine, it was necessary to superfuse it for 2 min. However, the response to histamine was prone to long-lasting tachyphylaxis (see Figure 1a). To try to avoid this, it was necessary to apply histamine for 1 min periods every 45 to 60 min. The 1 min application produced about 90% of the response to that of the 2 min superfusion. Although tachyphylaxis was not evident with this protocol, the response to a given concentration of histamine increased gradually throughout the day. Consequently, the effect of an antagonist on the histamine dose-response curve could only be realistically determined if the same protocol was performed during every experiment. The ganglia incubated with an antagonist could then be compared with untreated ganglia (see Methods). Without this methodology, it was only possible to assess whether an antagonist affected the response to one or two concentrations of histamine.

The slow depolarizing response to 300 μ M histamine (1 min) was not depressed by 1 μ M tetrodotoxin (*n* = 2) or by lowering the calcium chloride concentration in the medium to 0.1 mM (*n* = 4). This response was unaffected by a 30 min superfusion of the ganglion with propranolol or N-methylatropine (1 μ M, *n* = 4 each). It was also undiminished by the H₂ antagonists, cimetidine (10 μ M, *n* = 2) and metiamide (30 μ M, *n* = 4) and the H₂/H₃ receptor compounds impromidine (1 μ M, *n* = 4) and burimamide (10 μ M, *n* = 4). None of these compounds had a direct action by itself. In particular, the H₂ agonist impromidine did not evoke a direct response when superfused at 10 μ M for 1 min (*n* = 2). In contrast the H₁ antagonist mepyramine (1 μ M, 30 min) largely abolished the slow depolarization to 300 μ M histamine (see Figure 1b). The histamine receptor

Figure 1 The pharmacology of the depolarization induced by histamine (Hist), N²-methyl-histamine (N-Me), and 2-methyl-histamine (2-Me) on the rat superior cervical ganglion. Depolarization is upward in this and all subsequent figures. The agonist superfusions, indicated by the dots, were for 1 min. Histamine was superfused at 300 μ M unless otherwise indicated. Each line (a, b, c) depicts an experiment from a different ganglion: (a) shows that repeating the application of histamine at short intervals results in a smaller response, but after 2 h the response recovered. Line (b) shows that the response to 300 μ M histamine was abolished by mepyramine (Mep, 1 μ M, 30 min), and partial recovery was obtained after washing the ganglion for 3 h in mepyramine-free medium. Line (c) shows the relative potencies for evoking the slow depolarization of the three compounds: the superfused concentrations (in μ M) are shown.

agonists N²-methyl-histamine and 2-methyl-histamine also evoked the slow depolarization (see Figure 1c).

The dose-response curve to histamine is illustrated in Figure 2. It appeared to begin to level off at about 100–300 μ M, but clearly started to rise again at greater than 1 mM. Mepyramine (0.01, 0.1 and 1 μ M) antagonized the response to concentrations less than 300 μ M, but the responses to greater than 1 mM were less affected. The dose-response curves to 2-methyl-histamine and, in particular, N²-methyl-histamine were also biphasic (Figure 3). At concentrations less than 300 μ M

Figure 2 The concentration-response curve of the depolarization of the rat superior cervical ganglion to histamine (■). The effect of a 1 h incubation with different concentrations of mepyramine is also shown: 0.01 μ M (●); 0.1 μ M (○); 1 μ M (▲). Values are the geometric means ($\pm$ s.e.mean, shown by vertical bars) from 5 ganglia. Each response was expressed as a ratio of the response to 300 μ M histamine before the incubation period (see Methods).

Figure 3 Concentration-response curves of the depolarizations to histamine (■), N^α-methyl-histamine (○) and 2-methyl-histamine (◆) on the rat superior cervical ganglion. Accumulated data of the agonists were from 5, 7 and 6 different ganglia, respectively.

the rank order of potency of the three agonists was histamine > N^α-methyl-histamine > 2-methyl-histamine (see also Figure 1c), but with concentrations of over 1 mM the ranking was N^α-methyl-histamine > histamine ≈ 2-methyl-histamine.

mine. Mepyramine (10 nM) antagonized the responses to <300 μM of both N^α-methyl-histamine and 2-methyl-histamine to a similar extent to that seen with <300 μM histamine (not shown, $n = 4$ for each). An examination of the responses (Figure 4) revealed that a fast depolarization was evoked by high concentrations of histamine, and particularly by N^α-methyl-histamine.

Appropriate controls were sought when it was discovered that 3 mM histamine dihydrochloride reduced the pH of the superfusing medium from ca. 7.4 to ca. 7.0. (A similar drop in pH was noted with 3 mM N^α-methyl-histamine dihydrochloride). Control solutions were prepared by adding sufficient hydrochloric or sulphuric acid to similarly reduce the pH. When superfused for 1 min periods, these solutions evoked apparent fast depolarizations, but the responses were only 22–56% (median = 26, $n = 5$) of the response to 3 mM histamine recorded in the presence of 1 μM mepyramine. Furthermore, in experiments where the pH of the solutions containing either histamine or N^α-methyl-histamine (3 mM) was kept at 7.4 (by adding sufficient 0.5 M NaOH) the fast depolarizing response was still present, although it was 33–40% smaller than that recorded without adjusting the pH ($n = 3$).

Given that the agonist pharmacology of the fast response appeared to be different from that of the smaller slow response, we determined the sensitivity of the response to 3 or 10 mM histamine to a range of antagonists. Using such high

Figure 4 The pharmacology of the fast depolarization evoked by histamine (Hist) and N^α-methyl-histamine (N-Me) on the rat superior cervical ganglion. In (a) is shown the action of mepyramine (1 μM) on the responses to two different concentrations of histamine; (b) shows the relative potency of N^α-methyl-histamine and histamine in evoking the fast response in the presence of 1 μM mepyramine. The gains of the traces on the bottom line were half of those on the top line. The micromolar concentration of the agonists are indicated.

Figure 5 The action of N-methylbicyculline (Bic, 100 μM, 40 min) on the fast ganglionic depolarization to histamine recorded in the presence of 1 μM mepyramine. The micromolar concentration of histamine is shown.

concentrations of histamine, we repeated the responses at intervals of 1 h or greater and superfused each antagonist for 30–45 min. The fast response was not reduced by the histamine antagonists cimetidine (10 μ M, $n = 4$), impromidine (1 μ M, $n = 4$) and burimamide (10 μ M, $n = 4$). It was also unaffected by N-methylatropine (1 μ M, $n = 8$), propranolol (1 μ M, $n = 4$) or mecamlamine (10 μ M, $n = 6$). At the present time, the only antagonists of this response are (+)-tubocurarine (100 μ M, $n = 6$, 0–25%) and N-methylbucuculline (100 μ M, $n = 7$, 30–45%, see Figure 5). At this concentration, the latter did not reduce the slow depolarization to 300 μ M histamine ($n = 4$). The reduction of the fast response by N-methylbucuculline was observed in the presence or absence of mepyramine (1 μ M).

By contrast with their action on the fast histamine response, the geometric mean IC_{50} values for mecamlamine and (+)-tubocurarine against DMPP were 0.7 and 30 μ M, respectively ($n = 4$ for each). N-methylbucuculline and (+)-tubocurarine antagonized the GABA-induced depolarization ($n = 4$) with IC_{50} s of 2 and 17 μ M, respectively. Finally, in contrast to the lack of action on the histamine-induced fast response, lowering the calcium in the medium (from 2.5 to 0.1 μ M) reduced the nicotinic depolarization to DMPP by 50–75% ($n = 4$).

Discussion

The principal finding of this study was that histamine (10–300 μ M) evoked a small, slow depolarization of the rat superior cervical ganglion that was sensitive to mepyramine. At concentrations greater than 1 mM it also induced a larger, faster depolarization which did not appear to be mediated by H_1 , H_2 or H_3 receptors. The rank order of potency of agonists to evoke the slow depolarization was different from that necessary to induce the fast depolarizing response.

The slow depolarization to histamine appeared to be a direct action on the ganglion cells since it was not reduced by tetrodotoxin or by reducing the calcium concentration in the medium, either of which would reduce synaptic transmission. Slow ganglionic depolarizations can also be evoked by muscarinic receptors and β -adrenoceptors, but this histamine action was unlikely to be mediated by these receptors because of its resistance to N-methylatropine and propranolol. Thus, this response was probably mediated by a direct action of histamine on histamine receptors.

The sensitivity of the slow depolarization to mepyramine (estimated pA_2 of 9) and the lack of effect of H_2 and H_3 antagonists indicated that it was mediated by H_1 receptors. Two other histamine analogues N^α-methyl-histamine and 2-methyl-histamine also evoked a slow mepyramine-sensitive ganglionic depolarization. They were about 3 and about 10 times less potent than histamine, respectively.

Relatively high concentrations of histamine also evoked a fast depolarization. This was initially considered to be a non-specific action since such concentrations of histamine dihydrochloride reduced the pH of the superfusing medium. Comparable pH controls did evoke similar fast depolarizations, but these responses could only account for ca. 25% of the response to histamine. In addition, the fast response was still present when the pH was kept at 7.4. This complication, however, should be borne in mind when considering the pharmacology of the histamine-induced fast depolarization, since the pH of the medium was not routinely restored to 7.4.

The peak of the dose-response curve for the fast depolarization was not determined, but it was clearly much larger than that of the slow depolarization. The rank order of potency of the agonists in producing this response was different from that for the slow depolarization. N^α-methyl-histamine was about 3 times more potent than the other 2 agonists which were approximately equipotent. This potential change was not only insensitive to muscarinic and β -adrenoceptor antagonists, but it was also unaffected by the H_1 , H_2 and H_3

antagonists tested. Mepyramine did reduce the peak of the fast response to these concentrations of histamine, but its IC_{50} (0.1–1.0 μ M) was clearly greater than that against the slow response evoked by lower concentrations of histamine (IC_{50} ca. 10 nM, see Figure 2). Indeed, the antagonism of the fast response could be explained partly by a reduction of the underlying slow response.

A fast depolarization of this preparation can be evoked by the activation of nicotinic, GABA_A and 5-HT₃ receptors, so we first considered whether histamine could be activating one of these receptors. It is known that histamine interacts with the nicotinic receptor at the neuromuscular junction, albeit in an apparently competitive antagonist manner (Ariyoshi *et al.*, 1985), but we tested for the possibility that it could activate a nicotinic response on this preparation. The resistance of this response to mecamlamine and the small effect of (+)-tubocurarine indicated that the opening of nicotinic receptor-linked ion channels was improbable. Furthermore, unlike the depolarization to the nicotinic agonist DMPP, the fast histamine response was not reduced by superfusing low calcium containing medium. The relative resistance of the fast histamine response to (+)-tubocurarine was very informative. In addition to its antinicotinic properties, it also antagonizes 5-HT₃ (Yakel & Jackson, 1988) and GABA_A (Simmonds, 1986) receptor-mediated responses, so the fast histamine response was unlikely to be mediated by such receptors. It was therefore surprising when another GABA_A antagonist, N-methylbucuculline, reduced this response; however, the concentration used (100 μ M) was well above its affinity for GABA_A receptors (pA_2 5.9, Simmonds, 1986) and its IC_{50} value against the GABA response on this preparation (2 μ M).

The pharmacology of the histamine-induced fast depolarization was distinct from that of H_1 , H_2 and H_3 receptors, but considering the problems of interpretation arising from the pH changes and the absence of a potent and selective antagonist, it would be premature to conclude that this response was mediated by 'H₄' receptors. N-methylbucuculline did reduce the fast response without blocking the underlying slow depolarization, but its apparent affinity was low. We know of no other fast response to histamine on mammalian tissues which has this agonist and antagonist pharmacology, but histamine has been shown to evoke fast chloride-mediated responses on fly photoreceptors (Hardie, 1989) and lobster olfactory receptor neurones (McClintock & Ache, 1989). It is possible that the fast histamine-induced response of the rat superior cervical ganglion is mediated by a similar mechanism, since the opening of chloride channels (e.g. by GABA, Adams & Brown, 1975) will depolarize these cells. Quite clearly, however, further experiments are necessary before any conclusions can be drawn.

It has been shown that H_2 receptors mediate a depression of transmission across the rabbit (Brimble & Wallis, 1973) and rat (Snow & Weinreich, 1987) superior cervical ganglion. We found no evidence for an H_2 -mediated change in the membrane potential of these cells: H_2 antagonists had no effect on the potential changes evoked by histamine and the H_2 receptor agonist, impromidine, evoked no response by itself. Consequently, our evidence would support the conclusion of Snow & Weinreich (1987) that the depression of transmission is likely to be mediated presynaptically.

In conclusion, a small slow depolarization to histamine was recorded from the rat superior cervical ganglion. This response was probably mediated via H_1 receptors. It could contribute to the facilitation of transmission previously observed on this preparation (Snow & Weinreich, 1987). High concentrations of histamine also evoked a large, fast depolarization, but the pharmacology of this response was unlike that of H_1 , H_2 or H_3 receptors.

We thank Dr Jan Skidmore at Smith, Kline and French for the kind supply of compounds. We are also grateful to Juliet Davis for her help.

References

- ADAMS, P.R. & BROWN, D.A. (1975). Actions of gamma-aminobutyric acid on sympathetic ganglion cells. *J. Physiol.*, **250**, 85–120.
- ARIYOSHI, M., HASUO, H., KOKETSU, K., OHTA, Y. & TOKIMASA, T. (1985). Histamine is an antagonist of the acetylcholine receptor at the frog endplate. *Br. J. Pharmacol.*, **85**, 65–73.
- ARRANG, J.-M., GARBARG, M. & SCHWARTZ, J.-C. (1983). Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. *Nature*, **302**, 832–837.
- ARRANG, J.-M., GARBARG, M., LANCELOT, J.-C., LECOMTE, J.-M., POLLARD, H., ROBBA, M., SCHUNACK, W. & SCHWARTZ, J.-C. (1987). Highly potent and selective ligands for histamine H_3 -receptors. *Nature*, **327**, 117–123.
- ASH, A.S.F. & SCHILD, H.O. (1966). Receptors mediating some actions of histamine. *Br. J. Pharmacol. Chemother.*, **27**, 427–439.
- BLACK, J.W., DUNCAN, W.A.M., DURANT, C.J., GANELLIN, C.R. & PARSONS, E.M. (1972). Definition and antagonism of histamine H_2 -receptors. *Nature*, **236**, 385–390.
- BRIMBLE, M.J. & WALLIS, D.I. (1973). Histamine H_1 - and H_2 -receptors at a ganglionic synapse. *Nature*, **246**, 156–158.
- CHRISTIAN, E.P., UNDEM, B.J. & WEINREICH, D. (1989). Endogenous histamine excites neurones in the guinea-pig superior cervical ganglion *in vitro*. *J. Physiol.*, **409**, 297–312.
- DAUM, P.R., DOWNES, C.P. & YOUNG, J.M. (1983). Histamine-induced inositol phospholipid breakdown mirrors H_1 -receptor density in brain. *Eur. J. Pharmacol.*, **87**, 497–498.
- GANELLIN, C.R. (1982). Chemistry and structure-activity relationship of drugs acting at histamine receptors. In *Pharmacology of Histamine Receptors* ed. Ganellin, C.R. & Parsons, M.E. pp. 10–102. Bristol: Wright.
- HARDIE, R.C. (1989). A histamine-activated chloride channel involved in neurotransmission at a photoreceptor synapse. *Nature*, **339**, 704–706.
- HEGSTRAND, L.R., KANOF, P.D. & GREENGARD, P. (1976). Histamine-sensitive adenylate cyclase in mammalian brain. *Nature*, **260**, 163–165.
- ISHIKAWA, S. & SPERELAKIS, N. (1987). A novel class (H_3) of histamine receptors on perivascular nerve terminals. *Nature*, **327**, 158–160.
- KRSTIC, M.K. (1988). Pressor response mediated via histamine H_1 -receptors of the guinea-pig sympathetic ganglia. *Neuropharmacology*, **27**, 1215–1219.
- McCLINTOCK, T.S. & ACHE, B.W. (1989). Histamine directly gates a chloride channel in lobster olfactory receptor neurons. *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 8137–8141.
- NEWBERRY, N.R. & PRIESTLEY, T. (1987). Pharmacological differences between two muscarinic responses of the rat superior cervical ganglion *in vitro*. *Br. J. Pharmacol.*, **92**, 817–826.
- SUBRAMANIAN, N., WHITMORE, W.L., SEIDLER, F.J. & SLOTKIN, T.A. (1980). Histamine stimulates brain phospholipid turnover through direct H_1 receptor-mediated mechanism. *Life Sci.*, **27**, 1315–1320.
- SIMMONDS, M.A. (1986). Classification of inhibitory amino acid receptors in the mammalian nervous system. *Med. Biol.*, **64**, 301–311.
- SNOW, R.W. & WEINREICH, D. (1987). Presynaptic and postsynaptic effects of histamine and histamine agonists in the superior cervical ganglion of the rat. *Neuropharmacology*, **26**, 743–752.
- TRENDELENBURG, U. (1954). The action of histamine and pilocarpine on the superior cervical ganglion and the adrenal gland of the cat. *Br. J. Pharmacol. Chemother.*, **9**, 481–487.
- TRENDELENBURG, U. (1957). The action of histamine, pilocarpine and 5-hydroxytryptamine on transmission through the superior cervical ganglion. *J. Physiol.*, **135**, 66–72.
- WATSON, P.J. (1970). Drug receptor sites in the isolated superior cervical ganglion of the rat. *Eur. J. Pharmacol.*, **12**, 183–193.
- YAKEL, J.L. & JACKSON, M.B. (1988). 5-HT₃ receptors mediate rapid responses in cultured hippocampus and a clonal cell line. *Neuron*, **1**, 615–621.

(Received February 8, 1991
Accepted February 26, 1991)