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5-HYDROXYTRYPTAMINE DECREASES THE SENSITIVITY OF NICOTINIC ACETYLCHOLINE RECEPTOR IN BULL-FROG SYMPATHETIC GANGLION CELLS

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SUMMARY

1. The post-synaptic effects of 5-hydroxytryptamine (5-HT) were examined in neurones of bull-frog sympathetic ganglia with intracellular micro-electrode and voltage-clamp recording techniques. Atropine (1 μM) was used to block the muscarinic cholinoceptors.

2. 5-HT reduced the amplitude of the fast excitatory post-synaptic potential (fast e.p.s.p.). 5-HT also reduced the mean amplitude of the miniature excitatory post-synaptic potentials (m.e.p.s.p.s) without affecting their frequency.

3. Voltage-clamp studies showed that 5-HT decreased in a dose-dependent manner the amplitude of the acetylcholine (ACh) current produced by ionophoretic application of ACh to sympathetic neurones.

4. The relationship between the log of the ACh dose, applied ionophoretically, and the peak ACh current (the dose–response curve) was examined in voltage-clamped neurones. 5-HT caused a parallel shift to the right of the dose–response curve for ACh. Analysis using a double reciprocal plot (Lineweaver–Burk plot) revealed that 5-HT increased the apparent dissociation constant (K_m) of ACh for the receptor without changing the maximum ACh current (V_{max}), suggesting a competitive antagonism.

5. The relationship between the 5-HT dose and the magnitude of inhibition of the ACh current was obtained using two different amplitudes for the ACh response. The dose–response curve of 5-HT-induced inhibition using a relatively high amplitude ACh current, S_1, was parallel with that for a relatively low amplitude ACh current, S_2. The Dixon plot of these two curves yielded an apparent inhibition constant (K_i) of 42 μM.

6. Both fast excitatory post-synaptic currents (fast e.p.s.c.s) and miniature excitatory post-synaptic currents (m.e.p.s.c.s) had single-exponential decay time courses. The time constants of fast e.p.s.c. decay (τ_e) and m.e.p.s.c. decay (τ_m) were not altered by 5-HT, suggesting that 5-HT does not change the kinetics of opening and closing of the ionic channel associated with the nicotinic receptor. 5-HT did not alter the reversal potential of the fast e.p.s.c.

7. These results suggest that 5-HT decreases the sensitivity of the nicotinic receptor of sympathetic neurones, by interfering with ACh binding at the active site on the receptor–ionic-channel complex. 5-HT may physiologically inhibit cholinergic

transmission as it is an endogenous substance which antagonizes the nicotinic receptor in post-ganglionic neurones of bull-frog sympathetic ganglia.

INTRODUCTION

Since Trendelenburg (1957) observed the stimulant action of 5-hydroxytryptamine (5-HT) on the superior cervical ganglia, electrophysiological evidence has been accumulated to suggest a biological role for 5-HT in transmission through peripheral autonomic ganglia (Bindler & Gyermek, 1961; Hertzler, 1961; Gyermek & Bindler, 1962; de Groat & Volle, 1966; Jaramillo & Volle, 1968). Administration of 5-HT depolarized neurones in mammalian and amphibian sympathetic ganglia (de Groat & Lalley, 1973; Watanabe & Koketsu, 1973; Wallis & Woodward, 1974; Wallis & North, 1978). It was proposed that 5-HT is the mediator of the slow excitatory post-synaptic potential (slow e.p.s.p.) in enteric neurones (Wood & Mayer, 1979) and in some neurones of the coeliac–superior mesenteric plexus (Dun, Kiraly & Ma, 1984) of the guinea-pig. The existence of 5-HT has been demonstrated in the neurones of superior cervical sympathetic ganglion of the rat (Verhofstad, Steinbusch, Penke, Varga & Joosten, 1981) and the coeliac ganglion of the guinea-pig (Schultzberg, Hokfelt, Lundberg, Dalsgaard & Elfvin, 1983; Dun *et al.* 1984).

It is known that acetylcholine (ACh) mediates cholinergic nicotinic transmission in sympathetic ganglia, resulting in the fast excitatory post-synaptic potential (fast e.p.s.p.) (Eccles & Libet, 1961; Koketsu, 1969; Libet, 1970). The interaction of ACh with nicotinic receptors leads to the transient opening of receptor-coupled channels and produces a synaptic current. The amplitude of the post-synaptic current appears to depend on two factors: (1) the amount of ACh released from the preganglionic nerve terminals into the synaptic cleft and (2) the sensitivity of the nicotinic receptors of the post-synaptic neurones (cf. Koketsu, 1984). Electrophysiological experiments have shown that 5-HT modulates the release of ACh from the presynaptic nerve terminals in vertebrate sympathetic ganglia (Hirai & Koketsu, 1980; Dun & Karczmar, 1981)

Colomo, Rahamimoff & Stefani (1968) described a unique post-synaptic action of 5-HT at the frog skeletal muscle end-plate. 5-HT depressed the end-plate potential without changing the membrane potential and resistance. Subsequently, it was suggested that 5-HT decreased the sensitivity of the end-plate receptor (Akasu, Hirai & Koketsu, 1981; Akasu, Karczmar & Koketsu, 1983), presumably by blocking a binding site for ACh on the nicotinic receptor (Koketsu, Akasu, Miyagawa & Hirai, 1982). In the present paper, the effects of 5-HT on cholinergic transmission in bull-frog sympathetic ganglia were analysed with intracellular and voltage-clamp recording techniques. The results suggest that 5-HT inhibits nicotinic transmission post-synaptically by decreasing the sensitivity of the nicotinic receptors in a competitive manner. A brief report has been published previously (Akasu *et al.* 1981).

METHODS

Paravertebral sympathetic chains were isolated from bull-frogs (*Rana catesbeiana*) weighing 300–400 g. The eighth or ninth ganglion was superfused with Ringer solution. The method used for intracellular recordings with micro-electrodes was similar to that described in a previous paper

(Nishi & Koketsu, 1960). The fast e.p.s.p.s were elicited by stimulation of the preganglionic nerve fibre. In some experiments, preparations were soaked with a Ringer solution containing curare (3 μM) to reduce the amplitude of the fast e.p.s.p. The superfusing solution always included atropine (1 μM) to block the muscarinic receptors (cf. Derkach, Selyanko & Skok, 1983). The ACh-induced post-synaptic current (ACh current) was produced by ionophoretic applications of ACh to the surface of the ganglion cells from micropipettes filled with 1 M-ACh. ACh was applied every 10 s with rectangular current pulses with a duration of 10–100 ms. To prevent leakage of ACh from the pipette, weak anodal direct current of a few nanoamps was applied. The tip resistance of the ACh pipette was usually 70–150 MΩ. To obtain the dose–response curve for the ACh current, ACh was applied ionophoretically to a single ganglion cell according to the method described by Dreyer, Peper & Sterz (1978). The distance between the tip of the pipette and the ganglion cell surface could be estimated from the time to peak of the ACh current (80–120 ms) in the control conditions.

The method for voltage-clamp measurements was similar to that described by Takeuchi & Takeuchi (1959) (see also Kuba & Nishi, 1979; Akasu & Koketsu, 1981). Micro-electrodes were filled with 1 M-K citrate for injecting current. The feed-back amplifier was a Nihon Kohden CEZ-1100 with a maximum gain of 10000. The feed-back current was monitored with a current-to-voltage converter (mounted in CEZ-1100) that was connected to a Ag–AgCl electrode in the bath. The small residual fraction of the post-synaptic potentials which remained during voltage clamping amounted in amplitude to less than 1 % of the original potentials.

The ionic composition of the Ringer solution was as follows (mM): NaCl, 112; KCl, 2; $CaCl_2$ 1·8; and $NaHCO_3$, 2·4. All experiments were carried out at room temperature (22–24 °C). Drugs used were (+)-tubocurarine chloride (curare) (Sigma), atropine sulphate (Merck), acetylcholine chloride (Wako Pharmaceutical Co., Japan) and 5-hydroxytryptamine creatinine sulphate (Wako Pharmaceutical Co., Japan).

RESULTS

Fast e.p.s.p.

The resting membrane potential from sixty-three ganglion cells was $-58{\cdot}1 \pm 7{\cdot}3$ mV (mean $\pm$ S.D., $n = 18$). Stimulation of the preganglionic nerve fibres at a rate of 1 Hz elicited the fast e.p.s.p.s and associated action potentials in sympathetic neurones (Fig. 1). In B neurones of bull-frog sympathetic ganglia (Nishi, Soeda & Koketsu, 1965), 5-HT (5 μM–2 mM) had no appreciable effect on the resting membrane potential and resistance. The amplitude of the fast e.p.s.p. was gradually reduced to less than 10 mV after applying 5-HT (100 μM) to the Ringer solution for 3 min, and the action potential evoked by the fast e.p.s.p. was blocked (Fig. 1). The depression reached a steady state which was sustained throughout the exposure to 5-HT. No obvious tachyphylaxis was seen in the inhibitory action of 5-HT on the fast e.p.s.p. The inhibitory effect of 5-HT was reversible. The fast e.p.s.p. gradually returned to the control amplitude within 5 min after 5-HT was removed from the superfusing solution. The magnitude of the depression produced by 5-HT (10–300 μM) was investigated from ganglionic B neurones that were pre-treated with curare (3 μM). An example of these experiments is shown in Fig. 2. At a concentration of 50 μM, 5-HT produced an approximately 62 % depression of the amplitude of the fast e.p.s.p. The dependence of this effect on 5-HT concentrations (10, 50 and 300 μM) is shown in Table 1. In some ganglionic C neurones (Nishi *et al.* 1965), 5-HT had a depolarizing action (Fig. 2), although the amplitude of the depolarization varied in individual neurones. The mean amplitude of the depolarization was $2{\cdot}8 \pm 1{\cdot}6$ mV ($n = 7$). The fast e.p.s.p.s of C neurones were also depressed to 48·7 % ($n = 3$) of the control amplitude by 5-HT (50 μM) (Fig. 2). The time course of the fast e.p.s.p. was not altered by 5-HT at these concentrations (Fig. 2).

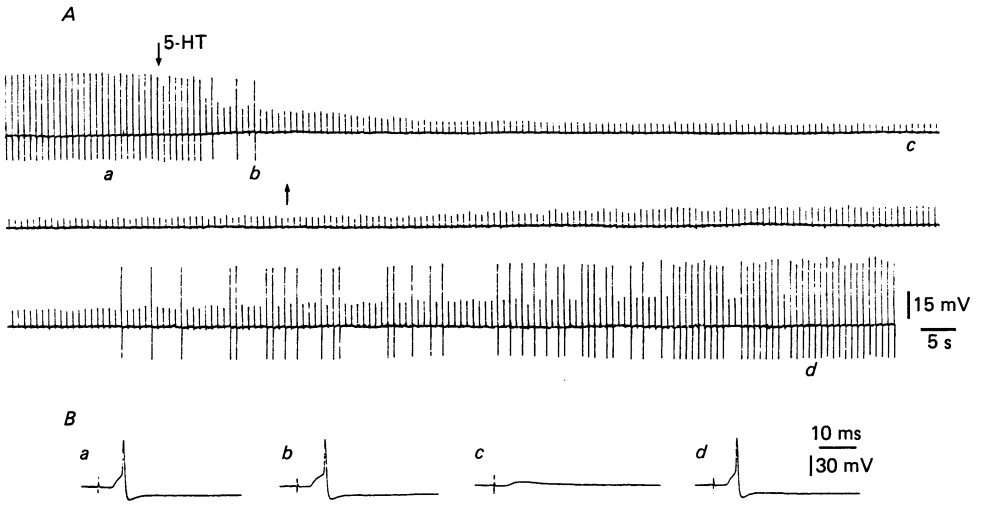

Fig. 1. Effects of 5-HT (100 μM) on the resting membrane potential and the responses evoked by stimulation (1 Hz) of preganglionic nerve fibres in sympathetic ganglia. Potential changes were recorded from a ganglionic B neurone. Only part of the action potential is shown in *A*, because a pen-writing recorder with a maximum frequency-response of 100 Hz was used. The period of 5-HT application is indicated by downward and upward arrows. *B*, oscilloscope recordings of the action potentials and fast e.p.s.p.s. Records *a*, *b* and *c* in *B* correspond to the times marked by the respective symbols in *A*. Note that the fast e.p.s.p. was markedly reduced by 5-HT, as shown in record *c*, where the action potential was blocked.

TABLE 1. Effect of 5-HT on the amplitude of the fast e.p.s.p.

Expt. 1	Control	10·8 ± 2·1 mV
(n = 8)	5-HT, 10 μM	9·2 ± 1·6 mV
	Ratio (5-HT/control)	0·80 ± 0·11
Expt. 2	Control	13·5 ± 1·3 mV
(n = 10)	5-HT, 50 μM	6·4 ± 0·8 mV
	Ratio (5-HT/control)	0·53 ± 0·10
Expt. 3	Control	13·1 ± 1·2 mV
(n = 9)	5-HT, 300 μM	3·5 ± 0·9 mV
	Ratio (5-HT/control)	0·28 ± 0·05

Fast e.p.s.p.s were recorded from ganglionic B neurones in a solution containing curare (3 μM). The preparation was equilibrated for approximately 5 min in solutions containing 5-HT. The data are expressed as mean ± S.D. The number of experiments (n) is given in parentheses.

Miniature e.p.s.p (m.e.p.s.p.)

The effect of 5-HT (100 μM) on the m.e.p.s.p.s was examined using ganglionic B neurones to avoid changes in the membrane potential and resistance during 5-HT application. In normal K^+, the application of 5-HT produced no appreciable effect on the frequency of m.e.p.s.p.s, while it depressed the amplitude of the m.e.p.s.p.s (Fig. 3*A*). Fig. 3*B* shows the effect of 5-HT on the amplitude histogram of the

m.e.p.s.p.s. The mean amplitudes obtained from 100 m.e.p.s.p.s in the control were 1·22±0·08 mV and they were reduced to 0·92±0·02 mV in the presence of 5-HT. Thus, 5-HT (100 μM) produced a 25·4±3·3% (mean±S.D., five preparations) depression of m.e.p.s.p. amplitude. The effect of 5-HT on m.e.p.s.p. amplitude was readily reversible after washing with Ringer solution. These results strongly suggest a post-synaptic inhibitory mechanism for the effect of 5-HT on the fast e.p.s.p.

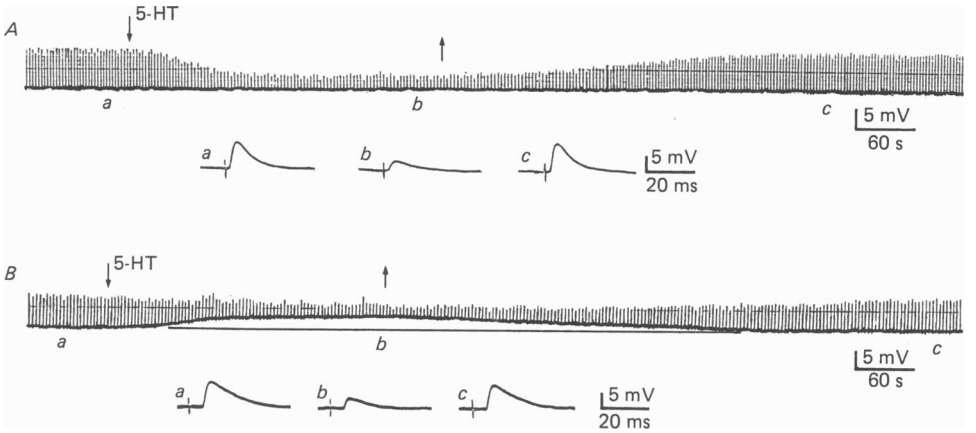

Fig. 2. Effects of 5-HT (50 μM) on the fast e.p.s.p.s recorded from the ganglionic B neurone (*A*) and C neurone (*B*) treated with curare (3 μM). The fast e.p.s.p.s were evoked by repetitive preganglionic nerve stimulations at a rate of 0·3 Hz. 5-HT was applied to the bath solution between the two arrows. In *A* and *B*, records *a*, *b* and *c* correspond to the times marked by respective symbols in upper traces.

ACh current

The post-synaptic mechanism of 5-HT action was examined directly with the ACh-induced synaptic current (ACh current) recorded by voltage-clamp techniques as an indicator of the sensitivity of the nicotinic ACh receptor. ACh was applied ionophoretically to the surface of the ganglion cells in a Ringer solution containing atropine (1 μM) where the muscarinic responses were eliminated (cf. Derkach *et al.* 1983). The amplitude of the ACh current was constant and reproducible when ionophoretic ACh pulses (2 nA for 80 ms) were applied at a rate of 0·1 Hz (Fig. 4). Bath application of 5-HT (5 μM–2 mM) produced only small (1–2 nA) or insignificant steady-state currents, while it strongly reduced the amplitude of the ACh current. One of these experiments is shown in Fig. 4. The application of 5-HT at a concentration of 100 μM caused an approximately 53% decrease in the amplitude of the ACh current. The depression reached the maximum level within 2 min which was sustained throughout the exposure to 5-HT (Fig. 4*A*). When 5-HT was removed from the superfusing solution, the amplitude of the ACh current returned to the control amplitude within 4 min. The time course of the ACh current was not altered by 5-HT (Fig. 4*B*). From eleven experiments, 5-HT at a concentration of 100 μM produced a 58·7±4·8% depression of the ACh current. The effect of 5-HT on the ACh

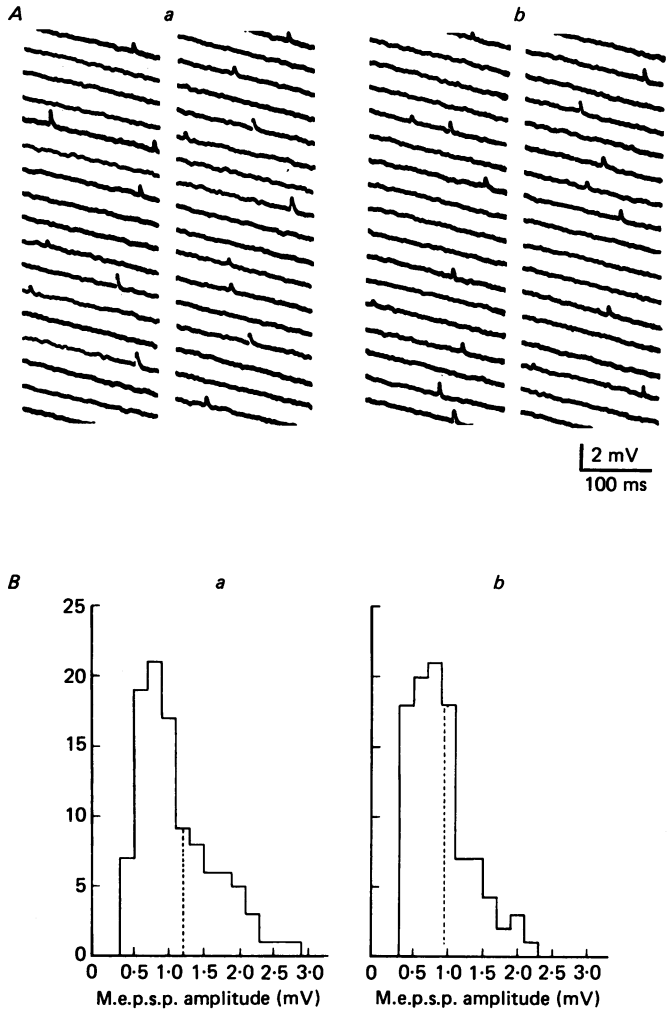

Fig. 3. Effect of 5-HT (100 μM) on the amplitude of m.e.p.s.p.s obtained from the B neurones. *A*, m.e.p.s.p.s obtained before (*a*) and 10 min after (*b*) application of 5-HT. *B*, amplitude histograms of m.e.p.s.p.s taken before (*a*) and after (*b*) the application of 5-HT. The ordinates indicate the number of m.e.p.s.p.s. Each histogram was constructed from 103 m.e.p.s.p.s. The mean amplitudes of the m.e.p.s.p.s are shown by the dashed lines. Mean amplitude of m.e.p.s.p.s for *a*, $1{\cdot}22 \pm 0{\cdot}08$ mV; for *b*, $0{\cdot}92 \pm 0{\cdot}02$ mV (mean $\pm$ S.D.).

current was dose dependent. Concentrations of 10 and 50 μM 5-HT caused a $13{\cdot}3 \pm 5{\cdot}3\%$ ($n = 6$) and $38{\cdot}3 \pm 3{\cdot}4\%$ ($n = 6$) decrease respectively in the amplitude of the ACh currents.

A similar inhibitory effect of 5-HT was observed on the response induced by ionophoretic application of carbachol which is resistant to the action of acetylcholinesterase. The depression of the ACh potential cannot be due to a decrease of the residual muscarinic response, since the slow inhibitory post-synaptic potential (slow i.p.s.p.) and slow e.p.s.p., which are mediated through muscarinic transmission, were

not affected by 5-HT. Creatinine sulphate (5 μM–2 mM) did not significantly affect the ACh current. These results indicate that 5-HT did depress the sensitivity of nicotinic ACh receptors of bull-frog sympathetic ganglion cells.

Interestingly, the magnitude of the 5-HT-induced depression was dependent on the size of the ACh current. Fig. 5*A* illustrates the effects of 5-HT (100 μM) on two ACh currents of different amplitudes which were recorded from the same neurone. An ACh current with an amplitude of 1·3 nA was produced by a relatively small

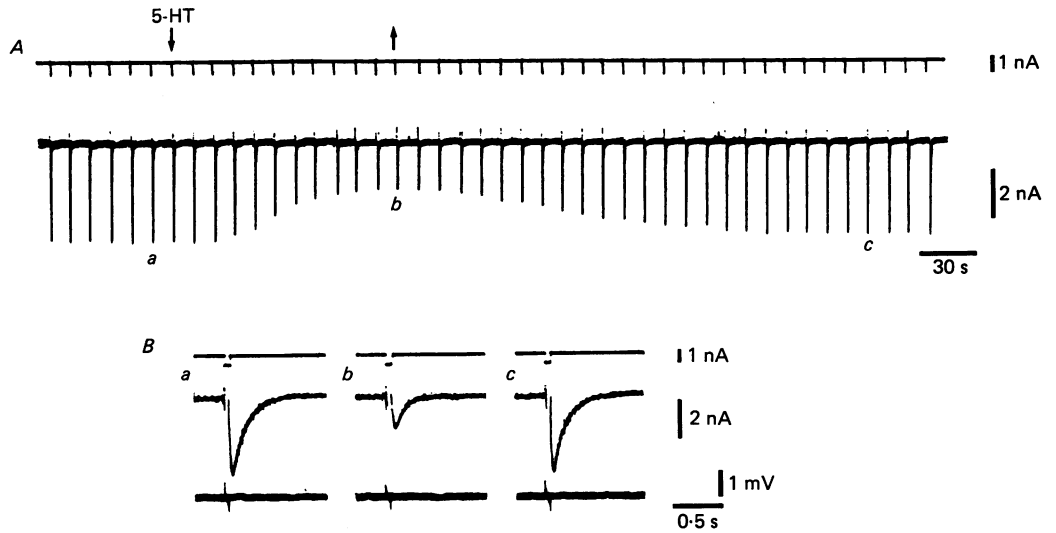

Fig. 4. Effect of 5-HT (100 μM) on the ACh current recorded from a voltage-clamped B neurone in the sympathetic ganglion. The membrane potential was held at − 60 mV. *A*, ACh currents were produced by ionophoretic pulses (0·5 nA for 50 ms) at a rate of 0·1 Hz. In *A*, the upper and lower traces indicate the current for ionophoresis and the ACh current, respectively. 5-HT was applied between the downward and upward arrows. *B*, oscilloscope recordings of ACh currents. Records *a*, *b* and *c* correspond to those indicated in record *A*. Upper traces indicate the currents for ionophoresis. Middle and lower traces indicate the ACh current and membrane potential clamped at − 60 mV, respectively.

ionophoretic pulse (6×10^{-11} C), while another ACh current (3·8 nA) was produced by a relatively high intensity pulse ($1{\cdot}9 \times 10^{-10}$ C). 5-HT (100 μM) produced a 70% depression of the low amplitude ACh current and a 40% depression of the high amplitude ACh current. Fig. 5*B* illustrates two different dose–response curves for 5-HT obtained using two different ACh currents as shown in Fig. 5*A*: the S_1 curve shows the relationship between 5-HT concentration and the amplitude of the ACh current (3·5 nA) induced by a relatively high concentration of ACh (2×10^{-10} C), while the S_2 curve for ACh current (1·2 nA) was obtained with a relatively low concentration of ACh (3×10^{-11} C). The S_1 curve appeared to be parallel to the S_2 curve. The concentrations of 5-HT which produced a half-maximal inhibition of the S_1 and S_2 curves were 0·07 and 0·13 mM, respectively (Fig. 5*B*). The inhibitory constant, a dissociation constant for the inhibitor–receptor complex (K_i), was evaluated from the relationship between 5-HT doses and reciprocal values of the ACh currents, the

Dixon plots of these two dose–response curves. A plot of the relative amplitude of the ACh current *versus* concentration of 5-HT at a high ACh concentration (S_1) yields a straight line as shown in Fig. 5*C*. Another series of experiments at a different concentration of ACh yielded a second straight line (S_2) with a different slope. The intersection of these two lines gives a K_i value. In this case, K_i was 42 μM.

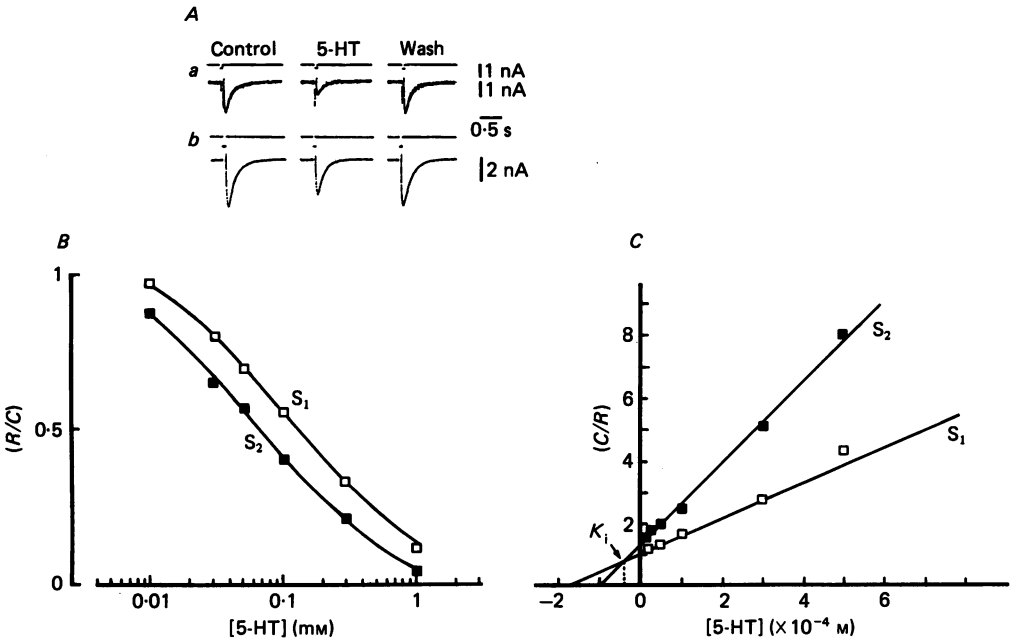

Fig. 5. *A*, effects of 5-HT (100 μM) on two ACh currents of different amplitude obtained from the same neurone. In *a*, the ACh current was produced by an ionophoretic pulse of 3×10^{-11} C. In *b*, the ACh current was produced by a 2×10^{-10} C pulse. In each record, the upper and lower traces indicate the current for ionophoresis and the ACh-induced current, respectively. 5-HT was applied for 5 min. Note that 5-HT is more effective on the ACh current with a small amplitude than on the current with a high amplitude. *B*. the relationship between the log of the 5-HT dose and the relative amplitude (*R*/*C*) of the ACh current, where *C* represents the amplitude of each control ACh current obtained in the Ringer solution and *R* represents the amplitude of the ACh current obtained in the presence of 5-HT (5 μM–3 mM). The S_1 curve (□) was obtained by plotting the inhibition of a large ACh current (3·5 nA) induced by a relative high concentration of ACh (1×10^{-10} C) against the 5-HT doses, while the S_2 curve (■) was obtained from a small ACh current (1·2 nA) induced by a low concentration of ACh (3×10^{-11} C). The ordinate and abscissa indicate the amplitude ratio (*R*/*C*) and the amount of 5-HT in mM, respectively. 5-HT was applied in the bath solution. *C*, Dixon plot obtained from the dose–response curve in *B*. □ and ■ correspond to the respective symbols in graph *B*. The ordinate and abscissa indicate the reciprocal value of the relative amplitude of the ACh current (*C*/*R* ratio) and log of the 5-HT dose, respectively. The intersect of these two lines gives the dissociation constant (K_i) for the inhibitor (5-HT).

Dose–response analyses of 5-HT-induced depression

The reduction of receptor sensitivity was investigated with dose–response relationships based on Michaelis–Menten kinetics. The dose–response curve was determined from ACh currents produced by ionophoretic ACh pulses (Dreyer *et al.* 1978). Applications of ACh at intervals of 10 s did not appear to produce desensitization of the nicotinic receptor of sympathetic neurones. As shown in Fig. 6*A*, S-shaped dose–response curves were obtained by plotting the peak amplitude of the ACh

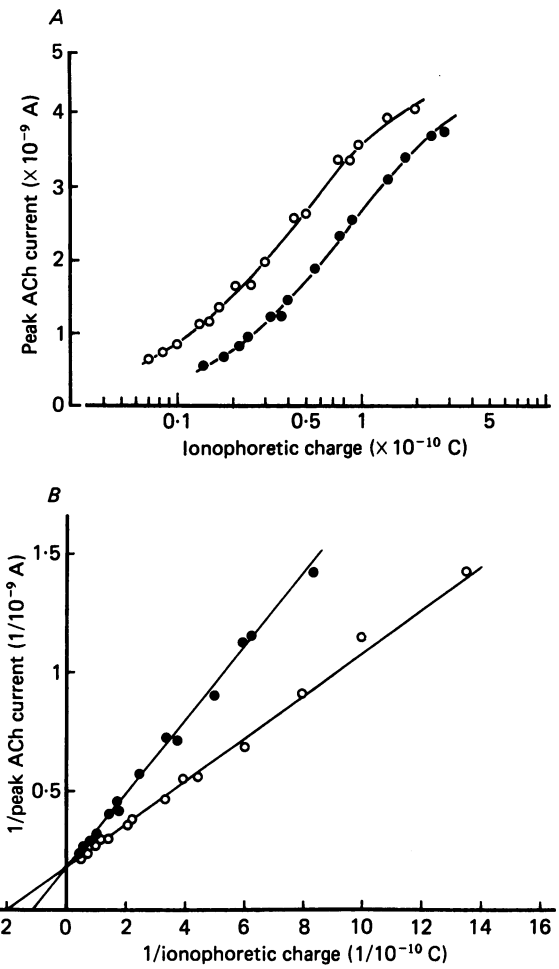

Fig. 6. *A*, the relationship between the log of the ACh dose (abscissa), applied ionophoretically to the ganglion cell and the peak amplitude of the ACh current (ordinate) obtained from a neurone voltage clamped at −60 mV. The amount of ACh applied to the cell was expressed as the electrical charge for ionophoresis. ○ and ● represent data obtained before and during the application of 5-HT (100 μM), respectively. *B*, a kinetic analysis using a double reciprocal plot (Lineweaver–Burk plot) constructed from *A* by assuming a Hill number (n_H) = 1. Note that 5-HT markedly decreased K_m, while it did not affect V_{max}. ○ and ●, as in *A*.

current against the quantity of ACh. 5-HT (100 μM) induced a parallel shift of this dose–response curve to the right (Fig. 6*A*), indicating a competitive inhibition by 5-HT. The relationship between the reciprocal of the ACh current peak and the amount of ACh (Lineweaver–Burk plot) yielded a straight line when the Hill number (n_H) was 1·0 (Fig. 6*B*). A straight line was also obtained in the presence of 5-HT. These two lines intersected at the ordinate. The ordinate intercept is indicative of the maximum ACh response (V_{max}). The apparent dissociation constant (K_m) for the ACh–receptor complex was increased by 5-HT. The kinetic parameters were evaluated; $K_m = 5$ nC, $K'_m = 7{\cdot}1$ nC, $V_{max} = 5$ nA and $V'_{max} = 5$ nA, where K_m and V_{max} were obtained in the control solution and K'_m and V'_{max} were obtained in the presence of 5-HT. These results indicated that 5-HT reduced the sensitivity, decreasing an apparent affinity of ACh to the binding site on the receptor–ionic-channel complex. This evidence supports the previous results obtained with current-clamp experiments (Akasu *et al.* 1981).

Reversal potential of the fast excitatory post-synaptic current (fast e.p.s.c.)

The fast e.p.s.c. (Kuba & Koketsu, 1978; Kuba & Nishi, 1979; MacDermott, Connor, Dionne &Parsons, 1980) was recorded from a voltage-clamped ganglion cell held at membrane potentials between + 40 and −80 mV (Fig. 7*A*). The amplitude of the fast e.p.s.c. increased as the membrane was hyperpolarized. When the membrane potential was decreased, the fast e.p.s.c. decreased in amplitude and finally reversed its polarity (Fig. 7*A*). Fig. 7*B* illustrates the relationship between the peak amplitude of the fast e.p.s.c. and the holding membrane potential over the range of −100 to + 40 mV. Linear amplitude–voltage relationships were obtained in the Ringer solution and in a Ringer solution containing 5-HT (100 μM). 5-HT depressed the fast e.p.s.c. by almost the same magnitude at all potentials. As shown in Fig. 7*B*, 5-HT (100 μM) produced no significant effect on the reversal potential of the fast e.p.s.c. The reversal potentials obtained in the control and in the presence of 5-HT (10 μM–2 mM) were $-8{\cdot}5 \pm 3{\cdot}3$ mV ($n = 13$) and $-9{\cdot}0 \pm 2{\cdot}9$ mV ($n = 13$), respectively.

Time course of the fast e.p.s.c. and the miniature e.p.s.c. (m.e.p.s.c.)

The effect of 5-HT on the time course of the fast e.p.s.c. was examined to test the possibility that the inhibition of the fast e.p.s.c. and ACh responses resulted from a shortening of the lifetime of these responses. Fig. 8*A* shows an example of the effect of 5-HT on the fast e.p.s.c. recorded at −60 mV. The rise time (time to peak) of the fast e.p.s.p. had a mean of $2{\cdot}1 \pm 0{\cdot}4$ ms which was not shortened in a solution containing 300 μM-5-HT. The decay of the fast e.p.s.c. followed a single exponential as indicated by a straight line relationship in the semilogarithmic plot of the fast e.p.s.c. amplitude against time. The mean decay time constant, τ_e, in nineteen neurones was $4{\cdot}8 \pm 0{\cdot}7$ ms at the holding membrane potential of −60 mV. 5-HT produced no appreciable change in τ_e (Fig. 8*A*). Table 2 also shows the decay time constants for the fast e.p.s.c. in control and in the presence of 5-HT at concentrations of 10, 100 and 300 μM. The decay time constant of the fast e.p.s.c. was dependent on the membrane potential (Kuba & Nishi, 1979; MacDermott *et al.* 1980). Fig. 8*B* illustrates the relationship between τ_e and the holding membrane potential. 5-HT

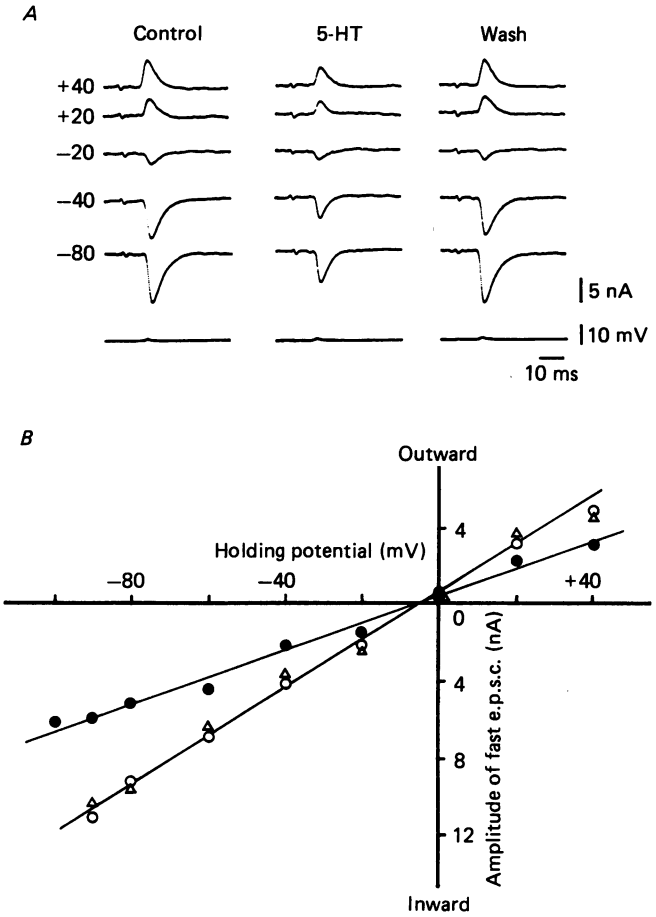

Fig. 7. *A*, effect of 5-HT (100 μM) on the fast e.p.s.c. obtained from a voltage-clamped B neurone. The holding membrane potentials are indicated in millivolts at the left column. The lowest traces are the residual fast e.p.s.p.s obtained at a holding potential of −80 mV. The fast e.p.s.c. was partially blocked by 3 μM-curare. The fast e.p.s.c.s in the left and middle columns were obtained before and 5 min after beginning the application of 5-HT (100 μM). The records in the right column were obtained 15 min after withdrawal of 5-HT. *B*, the current–voltage relationship for the peak amplitude of the fast e.p.s.c. (ordinate) and the holding membrane potential (abscissa). ○ and ● indicate the fast e.p.s.c.s before and 5 min after beginning the application of 5-HT (100 μM), respectively. △ indicates the fast e.p.s.c.s obtained 15 min after washing out the 5-HT.

(100 μM) did not produce a significant change in the voltage dependency of the decay time constant.

Similar experiments were performed using m.e.p.s.c.s. In the control solution, the mean amplitude of the m.e.p.s.c. from eighteen neurones was $0{\cdot}23 \pm 0{\cdot}03$ nA at a holding potential of −60 mV. In the presence of 5-HT (100 μM), the mean amplitude of the m.e.p.s.c. was reduced to $0{\cdot}14 \pm 0{\cdot}05$ nA, an estimated depression of approximately 39%. The m.e.p.s.c. had a single-exponential decay phase with a time

TABLE 2. Effect of 5-HT on the time constant of fast e.p.s.c. decay (τ_e)

Expt. 1	Control	4·5±0·8 ms
(n = 6)	5-HT, 10 μM	4·3±0·9 ms
Expt. 2	Control	4·2±0·5 ms
(n = 5)	5-HT, 100 μM	4·0±0·7 ms
Expt. 3	Control	5·1±1·2 ms
(n = 8)	5-HT, 300 μM	4·0±1·4 ms

Fast e.p.s.c.s were recorded from ganglionic B neurones in a solution containing curare (3 μM). The membrane potentials were held at −60 mV. The preparations were equilibrated for approximately 5 min in solutions containing 5-HT. The data are expressed as mean ±S.D. The number of experiments (n) is given in parentheses.

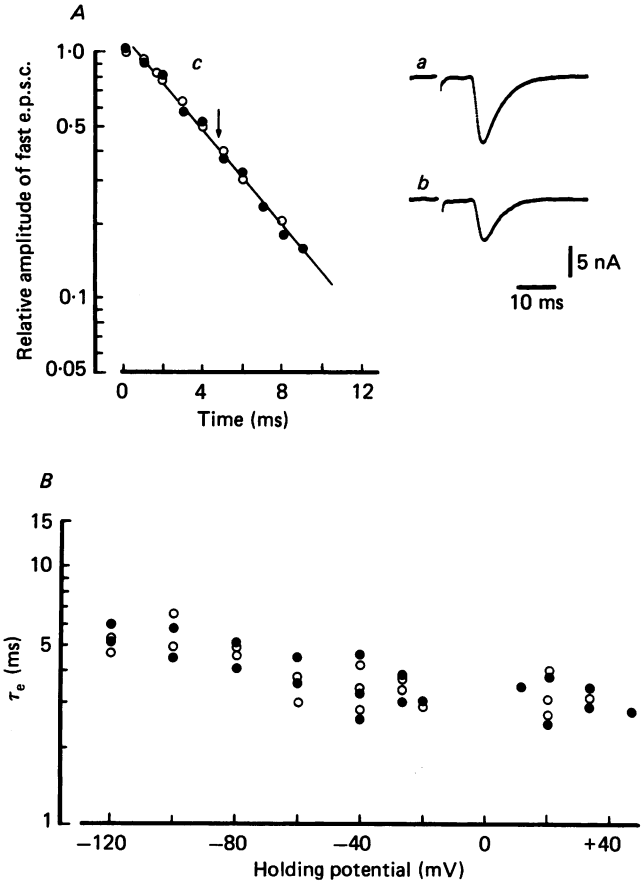

Fig. 8. Effect of 5-HT (100 μM) on the decay of the fast e.p.s.c. *A*, records *a* and *b* are oscilloscope recordings of fast e.p.s.c.s obtained before (*a*) and 5 min after beginning (*b*) the application of 5-HT. The holding membrane potential was −60 mV. The fast e.p.s.c. decays (*c*) were plotted on a log scale. The relative amplitudes of the fast e.p.s.c.s (ordinate) were plotted against time (abscissa), where the peak amplitude of each fast e.p.s.c. was expressed as 1. The arrow indicates the time constant of fast e.p.s.c. decay (τ_e). ○ and ●, before and after 5-HT applications, respectively. *B*, relationship between the time constants (τ_e) of decay for the fast e.p.s.c. (ordinate) and the holding membrane potential (abscissa) obtained from four ganglion cells. The ordinate is plotted on a log scale.

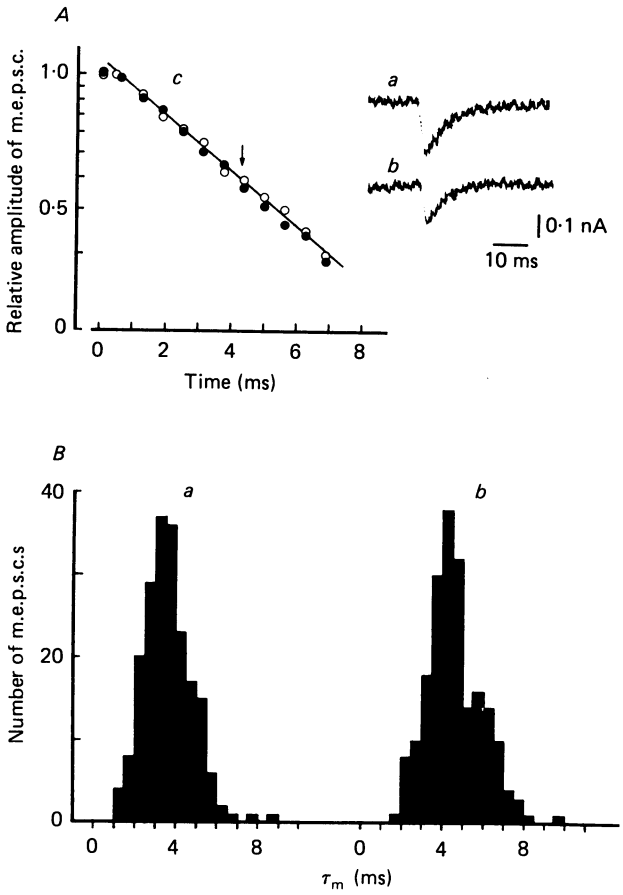

Fig. 9. Effect of 5-HT (100 μM) on the time course of m.e.p.s.c. decay. The holding membrane potential was −60 mV. *A*, m.e.p.s.c.s were recorded before (*a*) and 5 min after beginning (*b*) the application of 5-HT. The time course of the m.e.p.s.c. decay (*c*) is plotted on a log scale. The relative amplitudes of the m.e.p.s.c.s (ordinate) were plotted against time (abscissa), where the peak amplitude of each m.e.p.s.c. was expressed as 1. The arrow indicates the time constant of the m.e.p.s.c. decay (τ_m). ○ and ●, before and after 5-HT application, respectively. *B*, histograms of τ_m of the m.e.p.s.c.s calculated from 200 m.e.p.s.c.s. The ordinate and abscissa indicate the number of m.e.p.s.c.s and their time constant of decay, respectively. Histograms *a* and *b* were recorded in the control solution and in the presence of 5-HT (100 μM), respectively.

constant, τ_m, that was similar to τ_e. The value for τ_m measured in the control solution was 4·2 ms (Fig. 9*A*). The application of 5-HT at a concentration of 100 μM caused no change in the τ_m (Fig. 9*A*). Fig. 9*B* shows a histogram of the values for 200 m.e.p.s.c. recorded from six neurones. The τ_m values seem to have a Gaussian distribution. A similar unimodal distribution of τ_m has been shown to occur in neurones of the mammalian sympathetic ganglion by Derkach *et al.* (1983). The mean time constants of the m.e.p.s.c. decay were $4{\cdot}1 \pm 1{\cdot}3$ ms ($n = 18$) for the control and $3{\cdot}9 \pm 1{\cdot}4$ ms ($n = 18$) in the presence of 5-HT (100 μM). The difference of the two values was not statistically significant.

DISCUSSION

These experiments clearly demonstrate that 5-HT decreases the sensitivity of nicotinic receptors at the post-synaptic membranes of bull-frog sympathetic ganglion cells. Analysis of the dose–response curve for ACh currents indicated that 5-HT antagonizes the nicotinic ACh receptor in a competitive manner, decreasing an apparent dissociation constant (K_m).

The interaction of ACh with the nicotinic receptor leads to a transient opening of the receptor-coupled ionic channels, resulting in the generation of the synaptic current (Eccles & Libet, 1961; Koketsu, 1969; Libet, 1970). The competitive antagonists, curare and α-bungarotoxin, reduce the binding capacity of the end-plate receptors for the ACh released from nerve endings into the synaptic cleft (Katz & Miledi, 1973; Weber & Changeux, 1974). Kato, Kuba & Koketsu (1978) have reported for the skeletal muscle end-plate that erabutoxin B, a sea-snake venom, irreversibly blocks the binding of ACh to the receptor. 5-HT prevented this irreversible blockade by erabutoxin B, suggesting that 5-HT may combine with the specific binding site for ACh to prevent interaction of ACh with the nicotinic receptor–ionic channel complex (Koketsu *et al.* 1982). Kato, Kuba & Koketsu (1980) reported that erabutoxin B could block the ACh receptors in sympathetic ganglion cells, although the blockade was small and reversible. Furthermore, the electrophysiological properties of the nicotinic receptors in sympathetic ganglion cells are reported to be similar to nicotinic receptors at the end-plate (Kuba & Nishi, 1979; MacDermott *et al.* 1980). Thus, it seems likely that 5-HT acts by reducing the binding of receptor molecules with ACh. A simple representation of the reaction of 5-HT which competitively antagonizes the nicotinic receptor is shown below.

$$\begin{array}{ccccc} A + R & \overset{k_1}{\rightleftharpoons} & AR & \overset{\beta}{\rightleftharpoons} & AR^* \\ K_i \Big\| & \overset{k_2}{\uparrow} & & \underset{\alpha}{} & \\ 5\text{-HT} \cdot R & & & & \end{array} \quad .$$

A and R represent the free agonist (ACh) and the nicotinic receptor, respectively. AR is an intermediate, non-conducting agonist–receptor complex, and AR* is the activated conducting complex. k_1 and k_2 represent the rate constants for agonist binding to the receptor. The parameters β and α are the rate constants for channel opening and closing. K_i represents the dissociation constant for the 5-HT–R complex, 5-HT . R, obtained from the Dixon plot for the 5-HT action. The combination of 5-HT with the receptor–channel complex increases the amount of non-conducting complex 5-HT . R, which has no ability to open the ionic channel.

Since ACh current should be directly proportional to AR*, the actual density of the ACh current (I) can be described by following equation,

$$I = aNI_s, \tag{1}$$

where a is the probability that the ion channel is opened, N is the number of functional channels, and I_s is the ion current passing through a single channel. The current of a single channel (I_s) would be

$$I_s = I_g\,(V - E), \tag{2}$$

where I_g is the single-channel conductance, and E and V are the equilibrium potential for the ACh current and the resting membrane potential, respectively. 5-HT did not change the resting membrane potential or the reversal potential of the fast e.p.s.c. It is expected that the reduction of ACh current may be due to the decrease in the number of functional channels associated with the receptors or changes in the properties of a single ion channel, or both (cf. Koketsu, 1984). From the present results, 5-HT produced no changes in the decay time constants of the fast e.p.s.c. and the m.e.p.s.c. The voltage dependency of the time constant of the fast e.p.s.c. decay was not altered by 5-HT. It seems, therefore, unlikely that shortening of the channel lifetime is the main reason for the reduction of ACh current produced by 5-HT. We propose that 5-HT does not change the channel properties but decreases the number of functional receptor–ionic channel complexes. Analysis of single-channel current, I_s, with patch-clamp or noise analysis will provide direct evidence about 5-HT action on the single ion-channel properties.

The present result also shows that 5-HT depresses the amplitude of the fast e.p.s.p. Electrophysiological experiments have demonstrated that 5-HT modulates, dose dependently, the release of ACh from presynaptic nerve terminals in paravertebral sympathetic ganglia (Hirai & Koketsu, 1980). 5-HT at relatively high concentrations decreased the amount of ACh released from presynaptic nerve terminals (Hirai & Koketsu, 1980; Dun & Karczmar, 1981). Therefore, it appears that the depression of the amplitude of the fast e.p.s.p. results from both pre- and post-synaptic actions.

Since the existence of several types of 5-HT receptor has been postulated in central and peripheral nervous tissues (Haigler & Aghajanian, 1977; Fuller, 1980; Peroutka & Snyder, 1983), it is important to determine the specific 5-HT receptor mediating the depression of the nicotinic receptor. The present data, however, do not allow us to identify the type of 5-HT receptor involved. The receptor (or active site) for 5-HT on B type neurones does not seem to be directly coupled with a specific ionic conductance system, because 5-HT produced no changes in the membrane potential and conductance, but was closely related to the nicotinic receptor. Previous reports, demonstrating that curare blocked the 5-HT response (Gerschenfeld & Paupardin-Tritsch, 1974; Hirst & Silinsky, 1975; Higashi & Nishi, 1982), indicate a similarity of the affinity of receptors for ACh and 5-HT. A close association of the nicotinic and 5-HT receptors was also shown in superior cervical ganglion cells, where the blockade of one receptor species has often resulted in a potentiation of the responses mediated by the second receptor species (Nash & Wallis, 1980). Further investigations using several antagonists for the 5-HT receptor may characterize the nature of 5-HT receptor (or binding site) which suppresses the nicotinic receptor.

The physiological role of 5-HT in neurotransmission at paravertebral sympathetic ganglia is still unknown. A measurable amount of 5-HT has been reported to be present in the perfusate from the superior cervical ganglion (Gertner, Paasonen & Giarman, 1959). Immunohistochemical studies have demonstrated the existence of 5-HT-like immunoreactivity in sympathetic ganglia (Verhofstad *et al.* 1981; Schultzberg *et al.* 1983; Dun *et al.* 1984). Therefore, it is proposed that 5-HT is a possible endogenous substance which antagonizes the nicotinic ACh receptor of bull-frog sympathetic neurones.

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