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MODULATION OF SYNAPTIC TRANSMISSION AND EXCITATION–CONTRACTION COUPLING IN THE OPENER MUSCLE OF THE CRAYFISH, *ASTACUS LEPTODACTYLUS*, BY 5-HYDROXYTRYPTAMINE AND OCTOPAMINE

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SUMMARY

The modulatory actions of 5-hydroxytryptamine (5-HT) and octopamine (OA) were investigated in the opener nerve-muscle preparation of the crayfish, *Astacus leptodactylus*. Membrane resistance and resting potential were unaltered by 5-HT and OA at concentrations up to $2{\cdot}5 \times 10^{-5}$ M; but EPSP-amplitudes were increased, up to 3-fold by OA and up to 18-fold by 5-HT. The lowest effective concentration was $2{\cdot}5 \times 10^{-9}$ M; a maximal effect was produced at $2{\cdot}5 \times 10^{-6}$ M. The effect was reversible only after prolonged washing. The enhancement of EPSPs by 5-HT or OA is due to an increased amplitude of the synaptic current; the current duration is not altered. The facilitation ratio (ratio of amplitudes of a pair of EPSPs) is not significantly affected by 5-HT or OA despite the often enormous increase of the absolute EPSP-amplitudes.

The modulatory action also affects the excitation-contraction (e-c) coupling process: the effectiveness of e-c coupling was increased 7·4-fold by 5-HT ($2{\cdot}5 \times 10^{-6}$ M) and 18·7-fold by OA (5×10^{-6} M). The threshold potential of e-c coupling was not affected.

INTRODUCTION

Both 5-hydroxytryptamine (5-HT) and octopamine (OA) effectively enhance nerve-evoked contraction and EPSPs in crustacean skeletal muscle (Grundfest & Reuben, 1961; Dudel, 1965; Kravitz *et al.* 1976; Wheal & Kerkut, 1976; Florey & Rathmayer, 1978). For 5-HT a presynaptic mechanism of action has been deduced (Dudel, 1965), but experiments with 5-HT and OA also suggest postsynaptic effects which may be mediated by cAMP (Florey & Rathmayer, 1978; Batelle & Kravitz, 1978; Enyeart, 1981).

The present study was undertaken to further analyse the mechanism of action of these amines and to determine the relative extent of the involvement of presynaptic and postsynaptic effects. We address ourselves to the effects on membrane potential, membrane resistance, amplitude and time course of excitatory postsynaptic potential (EPSP) and excitatory postsynaptic current (EPSC), and on excitation–contraction coupling. Some of the results presented here have already been published in a short communication (Fischer & Florey, 1980).

Key words: 5-hydroxytryptamine, octopamine, excitation–contraction coupling.

MATERIALS AND METHODS

Astacus leptodactylus were obtained commercially. They were kept in slowly flowing lake water in shallow tanks at a temperature of 10 °C. The animals were fed with carrots and crayfish meat. The opener muscle, which is innervated by a single excitatory axon, was prepared from second and third walking legs as described earlier (Fischer & Florey, 1981).

The nerve-muscle preparation was mounted in a temperature-controlled muscle bath (temperature 10 °C, volume 1·5 ml) with the aid of small pieces of wax. The preparation was perfused with pre-cooled saline (10 °C) at a rate of 3 ml/min.

The saline used had the following composition: NaCl, 205 mM; KCl, 5·4 mM; $MgCl_2$, 2·6 mM; and $CaCl_2$, 13·5 mM. Addition of 10 mM-Tris hydrogen maleate (pH 7·2) was tried but found to be unnecessary since no obvious differences in performance or responses of the preparation were noticeable.

Appropriate concentrations of 5-HT (5-hydroxytryptamine creatinine-sulphate; Serva) and of OA (octopamine hydrochloride; EGA-Chemie) were made from stock solutions ($2·5 \times 10^{-3}$ M and 5×10^{-3} M, respectively).

The technique for recording and for current passing with the use of intracellular microelectrodes (3 M-KCl) was conventional. Nerve stimulation was accomplished with a suction electrode. Mechanical tension of the whole muscle or of single muscle fibres was recorded with a transducer tube (RCA 5734). Details of the experimental set-up have already been described (Fischer & Florey, 1981). The recorded electrophysiological parameters were fed into a computer system (Nicolet Med 80) for on-line analysis, storage and plotting. The programme was written in assembler language.

RESULTS

Nerve-evoked tension

5-HT caused a large increase of the tension evoked by nerve stimulation. As shown in Fig. 1A, the normally small tension produced by repetitive stimulation of the motor axon at 7 Hz for 2 s was greatly enhanced within 20 min of perfusion with $2·5 \times 10^{-8}$ M 5-HT. In the absence of 5-HT, stimulation of the motor axon with low frequencies (e.g. 2 Hz) rarely resulted in measurable contraction. After perfusion with 5-HT (e.g. $2·5 \times 10^{-7}$ M for 20 min) the same low rate of stimulation generated significant tension development (Fig. 1B); in fact even single stimuli produced measurable tension (see effect of first stimulus in Fig. 1B).

Like 5-HT, OA greatly enhanced the mechanical tension of the muscle due to nerve stimulation. Time course and magnitude of the effect were also similar to those seen with 5-HT application (see also Florey & Rathmayer, 1978).

Postsynaptic potentials (EPSPs) and postsynaptic currents (EPSCs)

The EPSPs evoked by repetitive stimulation characteristically showed considerable facilitation (for details see Linder, 1973). Application of either 5-HT or OA enhanced these EPSPs. In a typical experiment (Fig. 2), the peak amplitude of the fully

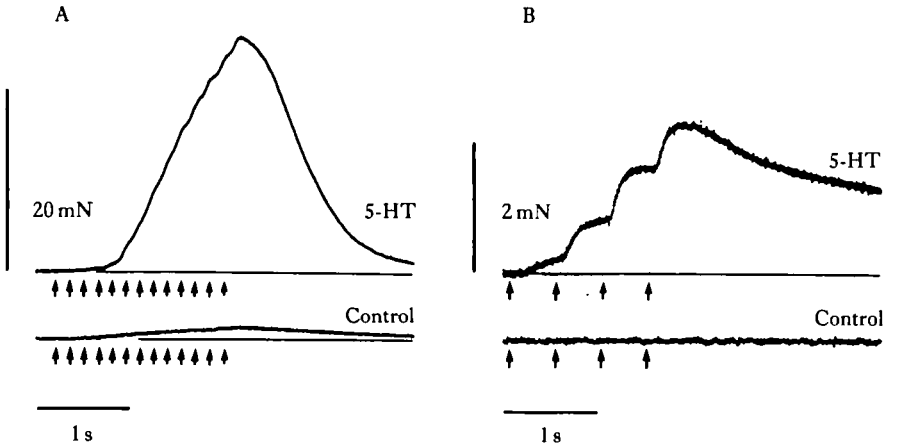

Fig. 1. The effect of 5-HT on nerve-evoked tension development. The example presented in A is typical for most cases; in B, an example of the extreme effectiveness of 5-HT is shown. Concentrations: $2{\cdot}5 \times 10^{-6}$ M in A and $2{\cdot}5 \times 10^{-7}$ M in B; stimulation frequencies are 7 Hz in A and 2 Hz in B. Arrows indicate stimulation pulses.

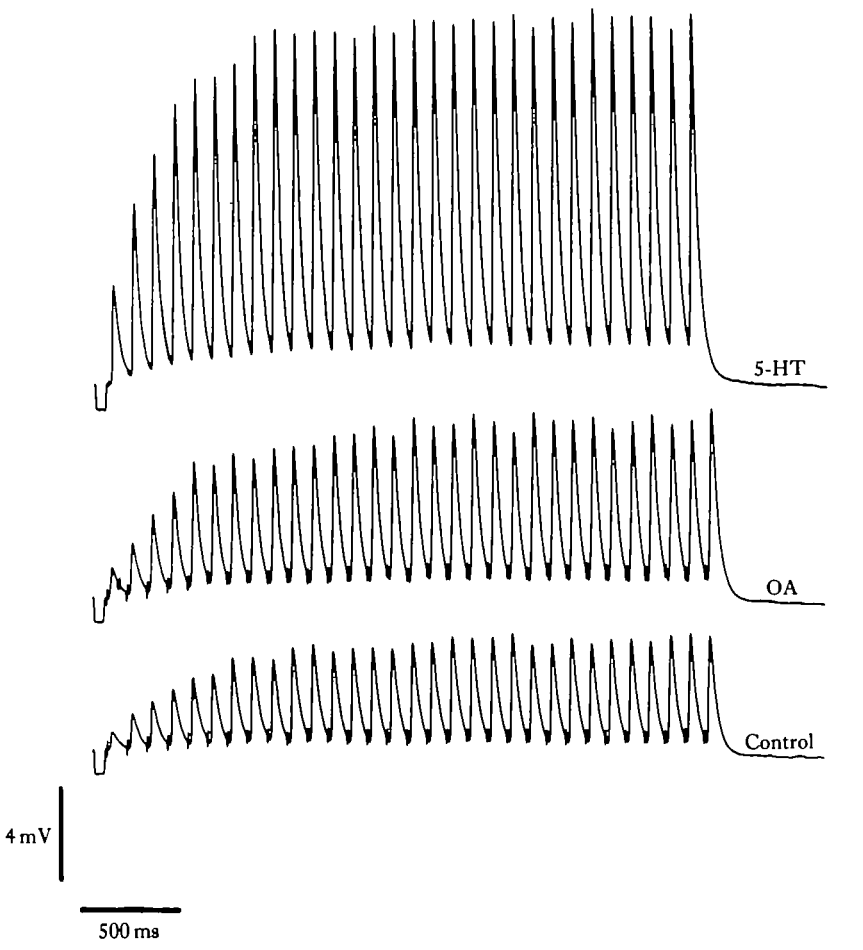

Fig. 2. The effect of OA and of 5-HT on EPSPs recorded from a muscle fibre during repetitive stimulation (10 Hz). Concentrations: 5×10^{-6} M (OA) and $2{\cdot}5 \times 10^{-6}$ M (5-HT).

facilitated EPSPs was increased 1·5 times by OA (5×10^{-6} M) and 3 times by 5-HT ($2{\cdot}5 \times 10^{-6}$ M). Similarly, there was a considerable enhancement of the first, unfacilitated EPSP of each train. Unfortunately, the amplitude of the small, unfacilitated and unenhanced EPSPs fluctuated considerably so that it is difficult to define ratios when only single trains are compared. For a more detailed analysis of 5-HT and OA action, a pair of EPSPs (100 ms interval) was evoked every 10 s and recorded on magnetic tape. The amplitude of the first, unfacilitated EPSP of each pair, the facilitation ratio (the quotient of the second and the first EPSP amplitude), and the time constant of the exponential phase of EPSP decay were then calculated. Increasing concentrations of 5-HT had a pronounced effect upon the unfacilitated EPSPs (Fig. 3). The effect was observable at concentrations down to $2{\cdot}5 \times 10^{-9}$ M (in some preparations the lowest effective concentration was $2{\cdot}5 \times 10^{-11}$ M) and reached a maximum at $2{\cdot}5 \times 10^{-6}$ M; no further augmentation occurred with higher 5-HT concentrations. The effect developed slowly and, once induced, persisted even if the preparation was washed. Particularly with the higher 5-HT concentration it took hours for the EPSP amplitude to return to the original level.

Because of this slow reversibility only cumulative dose-response curves (Fig. 4) could be obtained within the life-time of a preparation. A Hill plot of these data yields an n value of about 1.

From the data (see Fig. 3) it appears that with increasing 5-HT concentrations the facilitation ratio declines somewhat and reaches a constant level with those concentrations which yield maximal (saturating) amplitudes of the facilitated EPSPs. It would thus appear that 5-HT, by enhancing the amplitude of the first EPSP of each train (pair), pre-empts part of the initial facilitation process. This decline of the facilitation ratio is not always seen, however, and when it occurs it may well be the result of non-linear summation and of a non-linear relationship between transmitter action and EPSP amplitude.

The effects of OA on EPSP amplitude were qualitatively similar to those with application of 5-HT. However, OA was much less effective. The maximal effect of OA seen was a 3-fold increase, but the maximal effect of 5-HT was an 18-fold increase of EPSP amplitude! As shown in Table 1, the mean increase caused by an OA concentration of 5×10^{-8} M was only 1·12-fold but significantly differed from the control value. At the same low concentration of 5-HT the mean increase was 3·0-fold. At higher concentrations, OA sometimes caused a depression of EPSP amplitudes and generally did not enhance EPSPs beyond the effect seen at the low concentration of 5×10^{-8} M. The mean increases in these cases were not statistically significant. 5-HT, on the other hand, always caused an increase. At a 5-HT concentration of $2{\cdot}5 \times 10^{-5}$ M the mean increase was 6·4 times the control value.

5-HT was effective even after a maximally effective concentration of OA had been applied. A typical example is shown in Fig. 5: after application of 5×10^{-7} M OA, the amplitude of EPSPs was increased by 16% (already a maximal response!) but addition of 5-HT ($2{\cdot}5 \times 10^{-7}$ M) raised the amplitude to 360%. The same powerful action of 5-HT was noted whether or not OA (in concentrations as high as 5×10^{-4} M) was present. Although these results suggest independent receptors for 5-HT and OA we did not pursue this question further. (For insect muscle, Evans & O'Shea (1978) have provided evidence for separate receptors for 5-HT and OA.)

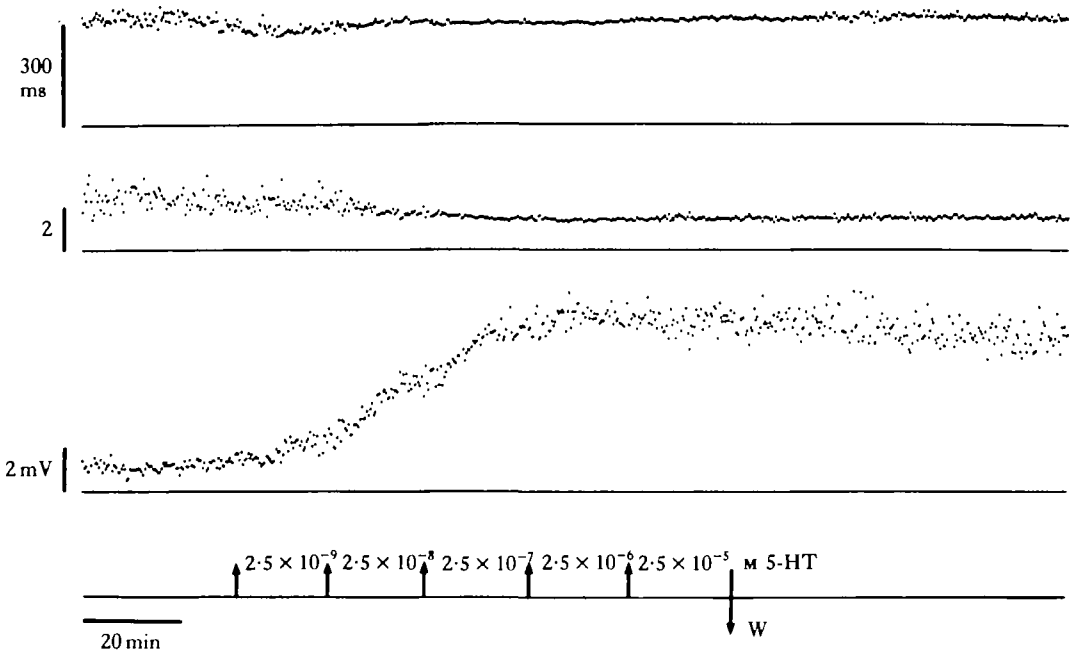

Fig. 3. Computer printout from a typical experiment in which increasing amounts of 5-HT were applied. Upper trace: membrane time constant; middle trace: facilitation ratio of the second and the first EPSP-amplitude (stimulation frequency 10 Hz); lower trace: amplitude of the unfacilitated EPSP. The reduction of variation of membrane time constant and of facilitation ratio with increasing 5-HT concentrations reflects the improvement of signal-to-noise ratio due to the increasing EPSP-amplitude. The increased scatter of the EPSP-amplitude after 5-HT application is a consistent feature but remains unexplained. W = washing.

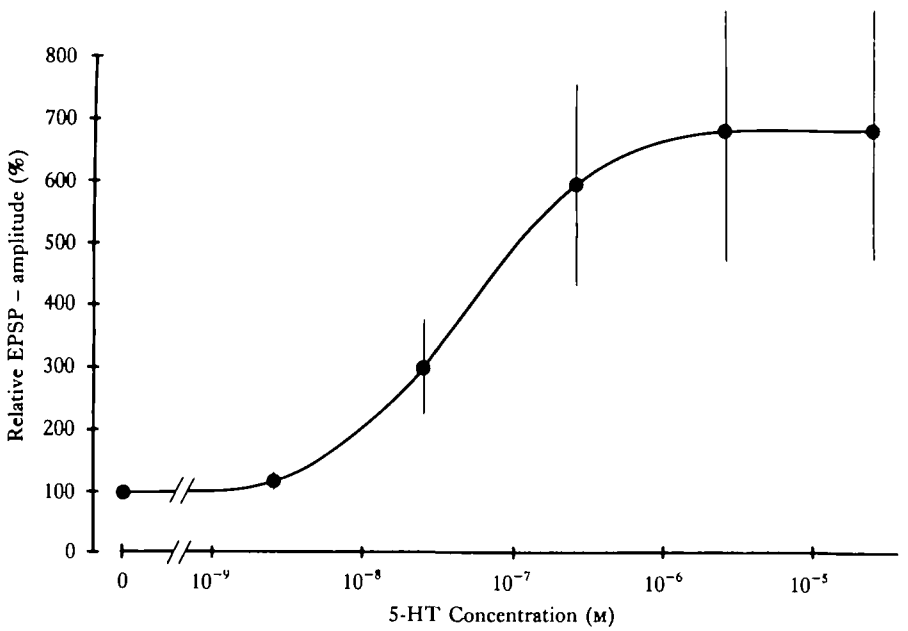

Fig. 4. Dose-response curve of the 5-HT effect on EPSP-amplitude. Mean values from six experiments; bars indicate S.E.

Table 1. *The effect of 5-HT (A) and of OA (B) on EPSP-amplitude, facilitation ratio (stimulation frequency 10 Hz), membrane time constant and resting potential*

Mean values ± S.D.; n = 15 for A and n = 19 for B. Values marked with ★ (★★) differ from the control value at a 1 % (0·1 %) significance level.

		EPSP amplitude (mV)	Facilitation ratio	Membrane time constant (ms)	Resting potential (mV)
A	Control	0·96 ± 0·71	1·96 ± 0·63	90·4 ± 82·3	−80·7 ± 7·9
	5-HT $2{\cdot}5 \times 10^{-8}$ M	★★2·90 ± 1·71 (+202 %)	1·86 ± 0·65 (−5 %)	92·2 ± 79·9 (+2 %)	−81·7 ± 7·7 (+1·2 %)
	5-HT $2{\cdot}5 \times 10^{-5}$ M	★★6·12 ± 3·94 (+538 %)	★1·78 ± 0·60 (−9 %)	98·6 ± 80·5 (+9 %)	−82·0 ± 7·4 (+1·6 %)
B	Control	0·86 ± 0·91	1·81 ± 0·35	162 ± 161	−81·4 ± 10·8
	OA 5×10^{-8} M	★0·96 ± 0·86 (+12 %)	1·84 ± 0·35 (+2 %)	168 ± 171 (+4 %)	−82·1 ± 10·3 (+1 %)
	OA 5×10^{-5} M	0·95 ± 1·00 (+11 %)	1·88 ± 0·36 (+1 %)	164 ± 193 (+1 %)	−81·6 ± 12·9 (+0·2 %)

5-HT and OA did not affect the passive electrical properties of muscle fibres; neither membrane potential nor membrane resistance (which is proportional to membrane time constant) was significantly altered even by high concentrations of the amines (Table 1).

In a recent publication Glusman & Kravitz (1982) argued that the effect of OA on EPSPs in lobster muscle could be explained by the observed increase in membrane resistance, implying that EPSP amplitude varies proportionally to membrane resistance changes. Lingle (1981) also emphasized the importance of the membrane resistance increase induced by dopamine (DA) in foregut muscles of crab and spiny lobster for the explanation of the enhancement of EPSPs. However, this argument, as we have shown (Fischer & Florey, 1982) by theoretical considerations and by experiments on crayfish muscle fibres, is incorrect. If the enhancement of EPSPs is not due to a resistance change it must be due to an increased amplitude and/or a prolonged duration of the underlying EPSCs.

A specially developed method of computing the synaptic current (Fischer & Florey, 1982) made it possible to evaluate the EPSCs from the corresponding EPSPs and to analyse the effects of 5-HT and of OA on amplitude and duration of EPSCs. (It is not possible to use the voltage clamp method with these large muscle fibres because their large membrane capacity introduces a time constant which prohibits effective clamping of the membrane potential within the short time intervals of the synaptic currents.) As illustrated in Fig. 5, both 5-HT, and to a much smaller extent OA, increase the amplitude of the synaptic current without altering its time constant. The lack of effect on the time constant was demonstrated by a computer programmed least square fit of the decay phase of the computed synaptic currents. The main effect responsible for the enhancement of EPSP amplitudes must be an increased transmitter output from the nerve terminals and/or an increased transmitter action. Experiments i

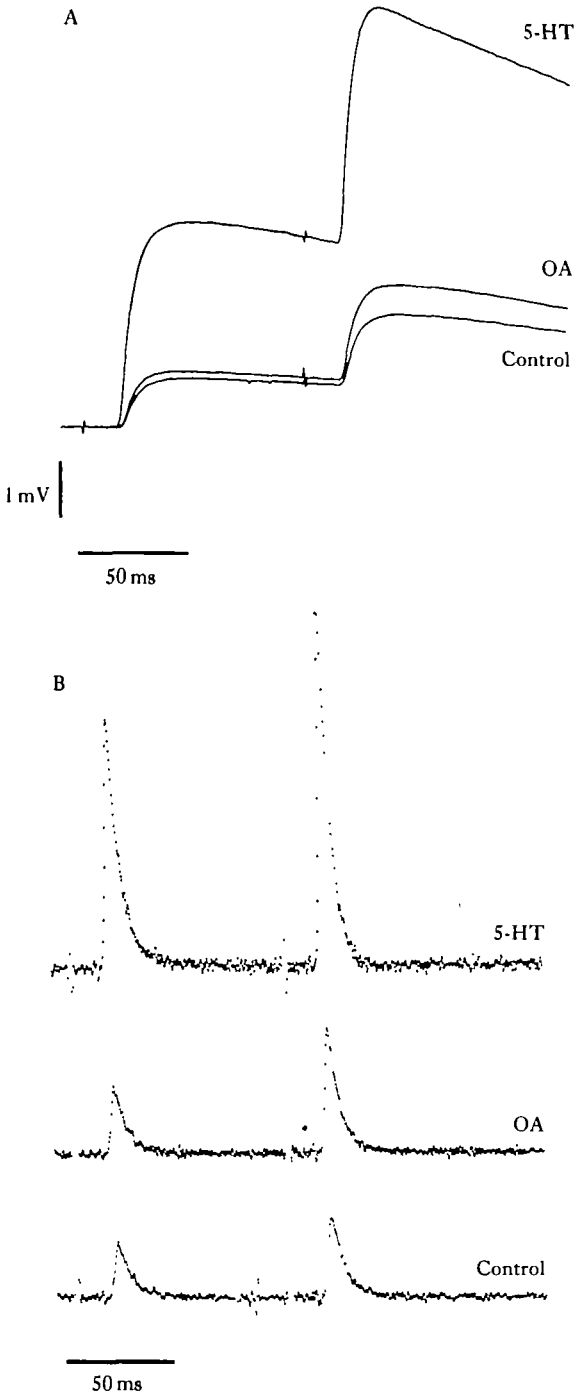

Fig. 5. Effect of OA and of 5-HT on averaged (n = 30) EPSPs (A), and on the synaptic currents (B) computed from them. OA (5×10^{-7} M) was applied before 5-HT ($2{\cdot}5 \times 10^{-7}$ M). Stimulation frequency was 10 Hz.

which the opener muscle was perfused during relatively short periods (10 s) with L glutamate (the motor transmitter) indicated no change of transmitter action by 5-HT.

Excitation-contraction coupling

Since the contractile responses to nerve stimulation were strongly enhanced by both 5-HT and OA, but only 5-HT caused a proportional increase of EPSP amplitudes, it seems likely that OA, if not 5-HT, exerts much of its action by affecting the contractile process itself.

To test for this we injected current into single muscle fibres with the aid of an intracellular microelectrode, and recorded the resulting tension first without and then in the presence of the respective amine in the bathing medium.

Within a few minutes after application of 5-HT or OA the amplitude of previously small contractile responses increased considerably (Fig. 6). With both amines, concentrations in the 10^{-6} M range already caused maximal responses. Typical examples are shown in Fig. 6.

The action of OA was more powerful than that of 5-HT. With both 5-HT and OA the dose-response relationship found for the action on directly stimulated contractions was similar to that already noted with regard to 5-HT and OA actions on EPSP- and EPSC amplitudes. With OA, however, the effects on the contractile events were usually much larger than those on EPSP (EPSC) amplitude.

In a series of experiments we determined the threshold for excitation-contraction coupling by subjecting muscle fibres to a graded series of depolarizations and noting the potential at which tension first began to appear. The preparations were then treated with 5-HT or OA and the threshold was again determined. There was no detectable change of excitation-contraction threshold with application of either compound. In a series of five experiments the mean threshold was $-57{\cdot}5\pm2{\cdot}7$ (S.D.) mV before, and $-57{\cdot}8\pm2{\cdot}6$ (S.D.) mV after application of $2{\cdot}5\times10^{-6}$ M 5-HT. Similarly, the mean threshold before and after application of OA (5×10^{-6} M) was $-55{\cdot}9\pm4{\cdot}4$ (S.D.) mV, and $-56{\cdot}0\pm4{\cdot}6$ (S.D.) mV respectively (seven experiments).

As has already been demonstrated (Orkand, 1962), the tension developed by crustacean (crayfish) muscle fibres is proportional to the suprathreshold depolarization. In a preceding publication we have shown that the coupling factor (which relates depolarization to tension) is temperature dependent (Fischer & Florey, 1981). We now find that 5-HT and OA strongly affect this coupling factor so that the slope of the curve which relates the magnitude of suprathreshold depolarization to the magnitude of the resulting contraction is increased conspicuously. In the experiment represented by Fig. 7, the tension produced by a depolarization pulse of 5 mV was enhanced 5-fold after application of $2{\cdot}5\times10^{-6}$ M 5-HT. In a series of seven experiments the tension (20–50 μN) generated by small suprathreshold depolarizations (2 s pulses) was increased by a factor of $7{\cdot}4\pm4{\cdot}2$ (S.D.) when a 5-HT concentration of $2{\cdot}5\times10^{-6}$ M was applied. With OA (5×10^{-6} M) there was an average increase of 18·7 times ($\pm14{\cdot}2$ S.D.) (3 experiments; range 9–35-fold).

Experiments with dopamine

In view of a recent report (Lingle, 1981) that dopamine (DA) enhances EPSPs and contractile responses of crustacean (*Cancer, Palunirus*) foregut muscles, we applied

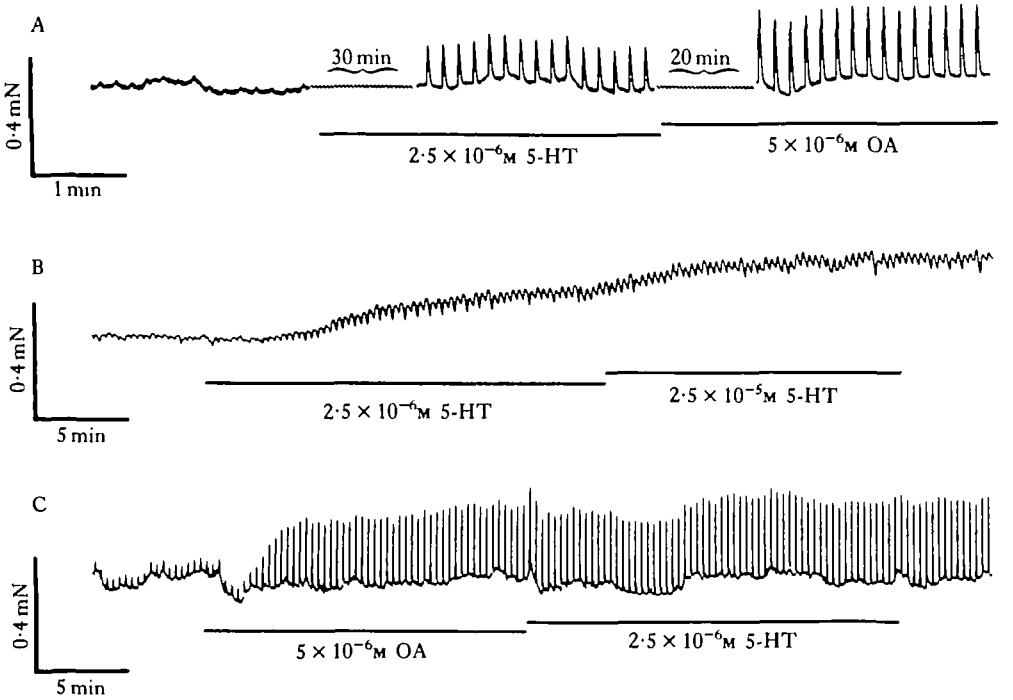

Fig. 6. The effect of 5-HT and of OA on tension induced by current injection into single muscle fibres. In A, the tension amplitude is enhanced about 10-fold; in B, 4-fold (the slow relaxation of tension results in a summation). In C, OA enhances tension amplitude 7-fold; the subsequent application of 5-HT induces no further augmentation.

DA (dopamine hydrochloride, EGA-Chemie) to the crayfish opener preparation. In none of the eight experiments were EPSPs or contractile responses to direct stimulation (single fibres) altered, even when higher concentrations (e.g. 5×10^{-5} M) were used.

DISCUSSION

The results of our investigation extend those of earlier studies concerning a presynaptic mechanism of action of 5-HT (Grundfest & Reuben, 1961; Dudel, 1965; Wheal & Kerkut, 1976). In particular we show that the increase of EPSP and EPSC is not accompanied by a significant change of the facilitation ratio. The enhanced EPSPs (EPSCs) are most likely the result of increased transmitter release. The possibility that they are caused by an increased affinity of the subsynaptic receptors for the motor transmitter (L-glutamate) has already been excluded by experiments of Wheal & Kerkut (1976) and of Kravitz *et al.* (1980), in which it was shown that 5-HT does not alter the effect of iontophoretically applied L-glutamate on crustacean skeletal muscle; our results confirm these observations.

Our investigation clearly demonstrates that both 5-HT and OA have powerful actions on the tension development induced by direct stimulation of single muscle fibres. Using extracellularly applied electrodes, Oota & Nagai (1977) found that in frog muscle tension is enhanced by catecholamines, and Weiss, Cohen & Kupfermann

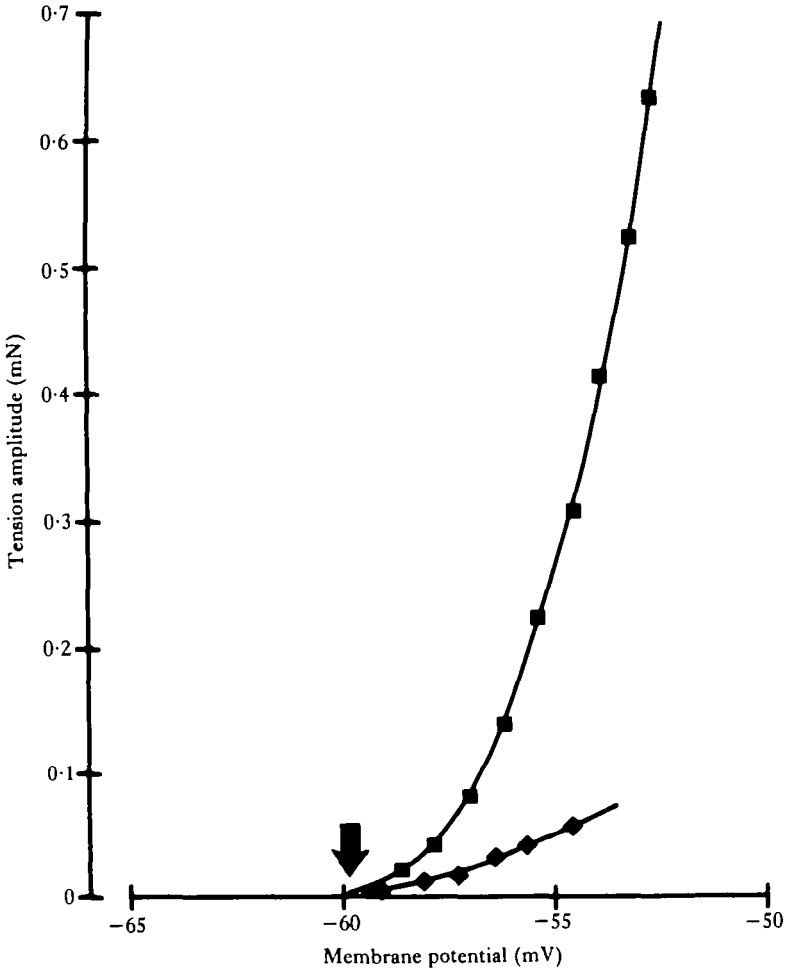

Fig. 7. 5-HT effect on tension amplitudes recorded from a single muscle fibre in relation to the membrane potential to which the fibre was depolarized by current injection for 2 s. The lower curve represents the state of responsiveness before 20 min application of $2{\cdot}5 \times 10^{-6}$ M-5-HT. The upper curve shows the state afterwards. The threshold of excitation-contraction coupling is indicated by an arrow.

(1978) reported enhancement by 5-HT of current-induced tension in snail muscle (although no experimental details were given). In experiments on locust muscle, O'Shea & Evans (1979) found that OA causes increased contractile responses. These authors did not employ direct stimulation of muscle fibres and could not decide whether part of the effect is a direct action on the muscle elements. All of the reported amine effects on contractile responses to direct or indirect stimulation were much weaker than those seen by us in our experiments on the crayfish opener muscle.

In the lobster the result of direct action of 5-HT and OA on opener muscle fibres is a contracture; DA relaxes the muscle (Kravitz *et al.* 1980). In the crayfish we found neither contracture nor relaxation effects induced by 5-HT, OA or DA.

The depolarization of muscle fibres up to and beyond the threshold potential of excitation-contraction (e-c) coupling is the first, initiated step in e-c coupling. It is possible that a simple shift of this e-c threshold towards the resting potential could

account for the enhancement of tension in directly stimulated muscle fibres even if all other parameters of e-c coupling are assumed to be unaltered. We can exclude this possibility for crayfish muscle fibres: the e-c threshold is affected neither by 5-HT nor by OA. At this stage it is not possible to decide which subsequent step (or steps) in the chain of events involved in e-c coupling is (or are) affected. An involvement of the Ca^{2+} activating mechanism is likely, however. The sarcoplasmic Ca^{2+}-pump may be stimulated as it was shown in frog muscle fibres to be the result of catecholamine action (Gonzales-Serratos, Hill & Valle-Aguilera, 1981). Enhancement of Ca^{2+} activation might also be the basis of the increased transmitter release from the terminals; Glusman & Kravitz (1982) recently suggested that a change in buffering or storage of Ca^{2+} is involved in the mechanism of 5-HT action in the nerve terminals of lobster muscle fibres.

In the case of OA the effect on e-c coupling is more powerful than that on transmitter release. Since the postsynaptic action of the amines is in series with the presynaptic effect one would indeed expect the effect on e-c coupling and that on transmitter release to be multiplicative. In a previous investigation (Florey & Rathmayer, 1978) it was found that with OA the contractions resulting from repetitive indirect stimulation could be nearly ten times greater than under control conditions, while EPSPs were enhanced at most 1·5 times. Results with 5-HT, like those in Fig. 1, can readily find their explanation in such a multiplication of pre- and postsynaptic effects.

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