J. Pharm. Pharmacol. 1988, 40: 855–861
Received March 8, 1988

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Activities of Octopamine and Synephrine Stereoisomers on Octopaminergic Receptor Subtypes in Locust Skeletal Muscle

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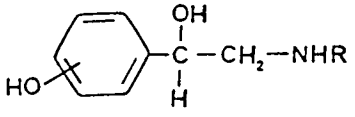
Abstract—The activities of the (−) and (+)- forms of *p*-, *m*- and *o*- octopamine and *p*- and *m*- synephrine have been compared on the different subtypes of octopamine receptor present in the extensor-tibiae neuromuscular preparation from the locust hindleg. The rank order of potency of the (−)-forms on the OCTOPAMINE$_{2A}$ receptors was *p*-synephrine > *p*-octopamine > *m*-octopamine > *o*-octopamine > *m*-synephrine whilst the rank order of the (+)-forms was *p*-synephrine > *p*-octopamine > *m*-octopamine. (+)-*m*-Synephrine and (+)-*o*-octopamine had no effect on this class of receptor when tested up to a concentration of 10^{-3}M. The rank order of potency of the (−)-forms on the OCTOPAMINE$_{2B}$ receptors was *p*-synephrine > *p*-octopamine > *m*-synephrine > *m*-octopamine > *o*-octopamine whilst the rank order of the (+)-forms was *p*-octopamine > *p*-synephrine > *m*-octopamine > *o*-octopamine. (+)-*m*-Synephrine again had no effect up to a concentration of 10^{-3}M. The rank order of potency of the (−)-forms on the OCTOPAMINE$_1$ receptors was *p*-synephrine > *p*-octopamine > *m*-synephrine > *m*-octopamine > *o*-octopamine, whilst the rank order of the (+)-forms was *p*-synephrine > *p*-octopamine > *o*-octopamine > *m*-synephrine > *m*-octopamine. In all cases the (−) enantiomers of all the structural isomers tested were more potent than the corresponding (+)-forms with isomeric activity ratios varying between 2 and 480. The activities of the octopamine and synephrine stereoisomers on the different octopamine receptor subtypes in the locust are compared with their published activities on the subtypes of mammalian α- and β-adrenoceptors.

The functional role of the biogenic trace amine, octopamine, in the vertebrate nervous system is at present enigmatic (Harmar 1980; Talamo 1980; Robertson 1981). Octopamine occurs in three different structural isomeric forms (*para-*, *meta-* and *ortho-*) and all of these have been shown to occur naturally in vertebrates (Ibrahim et al 1984; Williams et al 1984, 1987). The occurrence of *p*- and *m*- octopamine in the sympathetic nervous system (Ibrahim et al 1985) and brain (Danielson et al 1977; David & Delacour 1980) has led to the suggestion (Williams et al 1987) that they may function as co-transmitters or modulators of the action of noradrenaline. In addition the structural similarities between *m*- and *p*-octopamine and noradrenaline suggest that many of the observed physiological effects following the application of *p*-octopamine ('octopamine') to vertebrate tissues may be mediated via interactions with adrenergic receptors (see also Crowley et al 1983).



Isomeric octopamines and synephrines

Thus the actions of enantiomorphs of isomeric octopamines (R = H), and their *N*-methylated analogues, the synephrines (R = CH_3), have been examined on both α-adrenoceptor subtypes (Brown et al 1988) and β-adrenoceptor subtypes (Jordan et al 1987). In both cases, however, it was concluded that if *m*- and *p*-octopamine are coreleased with noradrenaline in amounts proportional to their concentration in tissue, then their activities on both α- and β-adrenoceptors would be too low for them to be considered physiologically significant. To date specific octopaminergic neurons have not been found in the vertebrate nervous system, although a differential distribution of octopamine has been reported in rat brain (Buck et al 1977). However, experimental evidence for the existence of specific octopaminergic receptors in vertebrates is limited to a very few papers in which single neurons in spinal cord, cerebral cortex (Hicks & McLennan 1978a, b) and thalamus (Dao & Walker 1980) have been shown to respond differentially to iontophoretic application of *p*-octopamine and noradrenaline.

In invertebrate nervous systems, on the other hand, specific octopaminergic neurons have been identified, together with target sites for the action of their released octopamine (see Evans 1980, 1985). Many of these target sites possess specific octopaminergic receptors with different pharmacological properties to those of vertebrate α- and β-adrenoceptors. The most intensively studied invertebrate preparation containing specific octopaminergic receptors is the extensor-tibiae muscle of the locust hind leg (Evans & O'Shea 1977, 1978; O'Shea & Evans 1979; Evans 1981). This preparation is innervated by an identified octopaminergic modulatory neuron and its octopamine receptors can be pharmacologically subdivided into a number of different subtypes (Evans 1981, 1987) with different modes of action (Evans 1984a,b,c). All the receptor subtypes have close pharmacological parallels with vertebrate α-receptor subtypes, but can be distinguished from the latter receptors since the octopamine receptors in the insect show a high preference

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for monophenolic biogenic amines. To date the only positional isomer of the octopamines or synephrine which has been identified unequivocally as endogenous in insects is *p*-octopamine; however, it has been claimed that the locust may be capable of synthesizing *m*-octopamine from L-dopa (David et al 1981). In view of this and our discovery that *o*-, *m*- and *p*-octopamine (together with *m*- and *p*-synephrine) are endogenous to vertebrates we are currently investigating the occurrence and biosynthesis of such compounds in invertebrates.

It is likely that endogenous octopamines and synephrines may arise via the same biochemical pathway (phenylalanine -> hydroxyphenylalanine -> hydroxyphenylethylamine -> hydroxyphenylethanolamine) (Brandau & Axelrod 1972; Boulton 1978; David & Coulon 1985). Hence, it would be logical to assume that each of these compounds may occur naturally as a single enantiomer. In view of this and the importance of the relationship between pharmacological activity and the chirality of asymmetric compounds (Ariëns 1986; Lehmann 1986) we report here the activities of the stereoisomers of *o*-, *m*- and *p*-octopamine and of *m*- and *p*-synephrine on the different subtypes of octopamine receptor present in the extensor-tibiae muscle preparation of the locust. This preparation contains both $OCTOPAMINE_2$ receptors that mediate their effects by increases in cyclic (c) AMP levels (Evans 1984a,b) and $OCTOPAMINE_1$ receptors that do not appear to mediate their effects via increases in cAMP levels, but which may act by releasing calcium from intracellular stores (Evans 1984c). The preparation does not, as far as is known, contain any adrenoceptors. A comparison of the results from the present study with those obtained by Brown et al (1988) on α-adrenoceptors and by Jordan et al (1987) on β-adrenoceptors may be of considerable value in future attempts to identify specific octopaminergic receptors in vertebrate preparations and to understand the physiological roles of *m*- and *p*-octopamine in vertebrates.

Materials and Methods

Adult locusts (*Schistocerca gregaria*) of either sex were obtained from crowded laboratory colonies fed on wheat seedlings. Small batches of animals were left for 1–2h before use, after removal from the main culture, to minimize any initial potentiation effects due to elevated levels of octopamine in the haemolymph (see Evans 1981; Davenport & Evans 1984a, b).

Tension in the extensor-tibiae muscle of the locust hindleg was measured almost isometrically with a tension transducer attached to the apodeme. The slow extensor-tibiae (SETi) motor neurone was excited at a frequency of 1Hz by stimulating nerve 3b with a pair of silver hook electrodes (O'Shea & Evans 1979). An operational amplifier signal differentiator was used to measure continuously the rates of neurally evoked contraction and relaxation (Buchan & Evans 1980).

The effects of drugs on the $OCTOPAMINE_{2A}$ receptors were measured as increases in the amplitude of SETi-induced twitch tension and the effects of drugs on the $OCTOPAMINE_{2B}$ receptors as increases in the rate of relaxation of SETi-induced twitch tension (Evans 1981). The drugs were dissolved in a physiological isotonic saline (pH 6·8, containing NaCl 140; KCl 10; $CaCl_2$ 4; $NaHCO_3$ 4; NaH_2PO_4 6; $MgCl_2$ 2; sucrose, 90mM) and superfused directly onto the surface of the muscle as 30s pulses (see O'Shea & Evans 1979). The effects of drugs on the $OCTOPAMINE_1$ receptors were assessed as the percentage reduction in the frequency of the myogenic rhythm of the extensor-tibiae muscle (Evans 1981) produced by the introduction of a 5-min pulse of the drug into the muscle superfusate. Desimipramine, a potent inhibitor of the high-affinity uptake of octopamine does not increase the responses of the preparation to octopamine or synephrine, indicating that uptake of the isomers and subsequent release of endogenous octopamine from nerve terminals does not affect the potency ratios determined in the present experiment (see Evans 1981).

Drugs

Drugs were obtained from the following sources: (−)-*m*-synephrine. HCl (m.p. 141–142°C, $[\alpha]_D^{22}$, −43°, c 0·1 (H_2O))—BDM Ltd; (+)-*m*-synephrine. HCl (m.p. 142°C, $[\alpha]_D^{22}$ + 50·3°, c. 0·1 (H_2O))—Ganes Chemicals Inc. Racemic *m*- and *p*-octopamine and *p*-synephrine were resolved with appropriate (+)- and/or (−)-organic acids, followed by fractional crystallization of the diasteroisomeric salts and ion-exchange to afford the corresponding optically active hydrochloride salt. Full experimental details of these procedures and determinations of the absolute configurations of these compounds will be published elsewhere. (±)-*m*-Octopamine. HCl (Aldrich Chem. Co. Ltd; (±)- and (−)- *o,o*-dibenzoyltartaric acid, Aldrich Chem. Co. Ltd) afforded (−)-*m*-octopamine. HCl (m.p. 127°C, $[\alpha]_D^{22}$ − 39°, c.0·1 (H_2O)) and (+)-*m*-octopamine. HCl (m.p. 125°C, $[\alpha]_D^{22}$ + 37·5°, c.0·1 (H_2O)). (±)-*p*-Octopamine. HCl (Aldrich Chem. Co. Ltd; (+)-10-camphorsulphonic acid monohydrate, Aldrich Chem. Co. Ltd) gave (−)-*p*-octopamine. HCl (m.p. 176°C, $[\alpha]_D^{22}$ − 50°, c.0·1 (H_2O)) and (+)-*p*-octopamine. HCl (m.p. 177–178°C, $[\alpha]_D^{22}$ + 46°, c.0·1 (H_2O)). (±)-*p*-Synephrine. HCl (Sigma; (+)- and (−)-bromocamphorsulphonic acid, ammonium salt, Aldrich Chem. Co. Ltd and Chemical Dynamic Corp., respectively) yielded (−)-*p*-synephrine. HCl (m.p. 176°C, $[\alpha]_D^{22}$ − 39°, c.0·1 (H_2O)) and (+)-*p*-synephrine. HCl (m.p. 178°C, $[\alpha]_D^{22}$ + 42°, c.0·2 (H_2O)).

Dose-response curves were obtained by applying pulses of drugs of increasing concentration to each preparation. Agonist potency was measured as the concentration required to produce 50% of the maximum response to (−)-*p*-octopamine (EC50 OCT) because, for some agonists, maxima could not be obtained due to an insufficient supply of test substances to complete the curves. The values obtained in these cases may not be the true EC50 values but it is important to retain these parameters to enable more meaningful comparisons to be made with previous work on vertebrate α- and β-adrenoceptors (Jordan et al 1987; Brown et al 1988). Calculation of potency was made by graphical interpolation of the curve for log (agonist concentrations) versus response to find the pD_2 OCT (−log agonist concentration) which gave 50% of the maximum response to (−)-*p*-octopamine. For individual tissues EC50 = antilog ($-pD_2$). The average potency for a given compound is expressed as the mean of the $-pD_2$ OCT values ± sem. In addition, for each drug, the relative potency and fraction of the maximum

response to that obtained for (−)-*p*-octopamine is given. For comparison with the results obtained in vertebrate preparations the results obtained for each drug are also expressed as their relative potency to the response obtained for (−)-noradrenaline.

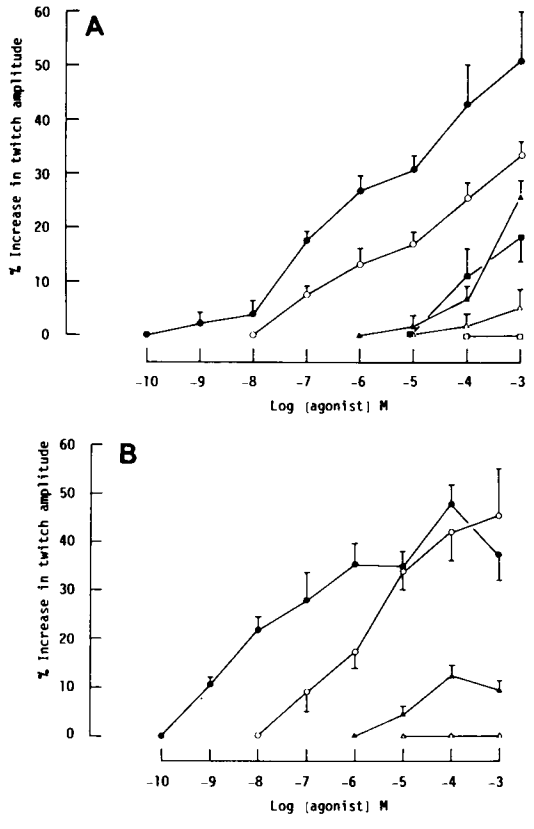

FIG. 1. The actions of the stereoisomers of octopamine (1A) and synephrine (1B) on the OCTOPAMINE$_{2A}$ receptors in the locust extensor-tibiae muscle. The results are plotted as the percentage increase in the amplitude of SETi-induced twitch tension against Log agonist concentration (M). ●(−)-*p*, ○(+)-*p*-, ▲(−)-*m*, △(+)-*m*, ■(−)-*o*-, □(+)-*o*-isomers.

Results

Effects on OCTOPAMINE$_2$ receptors modulating neuromuscular transmission

Neuromuscular transmission mediated by the slow excitatory motor neuron (SETi) to the extensor-tibiae muscle of the locust metathoracic hindleg is modulated by both presynaptic and postsynaptic receptors which have been designated OCTOPAMINE$_{2A}$ and OCTOPAMINE$_{2B}$ receptors, respectively (Evans 1981). Drugs that affect the OCTOPAMINE$_{2A}$ receptors increase the amplitude of SETi-induced twitch tension and drugs that affect the OCTOPAMINE$_{2B}$ receptors increase the rate of relaxation of SETi-induced twitch tension.

The activities of the (−)- and (+)-forms of *p*-, *m*- and *o*-octopamine on OCTOPAMINE$_{2A}$ receptors are shown in Fig. 1A and those of the (−)- and (+)-forms of *p*- and *m*-synephrine in Fig. 1B. The rank order of potency of the (−)-forms was *p*-synephrine > *p*-octopamine > *m*-octopamine > *o*-octopamine > *m*-synephrine. The activity of *p*-synephrine was almost seven times more than that of *p*-octopamine and that of *m*-octopamine was three orders of magnitude less than that of *p*-octopamine. At the highest concentrations tested (10^{-3}M) both *m*-synephrine and *o*-octopamine failed to produce increases that reached 50% of the increase produced by *p*-octopamine. The rank order of potency of the (+)-forms was similar with *p*-synephrine > *p*-octopamine > *m*-octopamine >> *m*-synephrine = *o*-octopamine. However, (+)-*m*- synephrine and (+)-*o*-octopamine did not produce any increases in twitch amplitude when tested at concentrations up to 10^{-3}M. The mean EC50 values and average potency values for these compounds for OCTOPAMINE$_{2A}$ receptors are presented in Table 1 together with their isomeric activity ratios. The (−)-isomers are the most active on the OCTOPAMINE$_{2A}$ receptors, whereas the (+)-isomers are up to two orders of magnitude weaker.

The activities of the (−)- and (+)-forms of *p*-, *m*- and *o*-octopamine on OCTOPAMINE$_{2B}$ receptors are shown in Fig. 2A and of the (−)- and (+)- forms of *p*-synephrine and *m*-synephrine in Fig. 2B. The rank order of potency for the

Table 1. The activity of octopamine and synephrine stereoisomers on OCTOPAMINE$_{2A}$ receptors in locust extensor-tibiae muscle.

	N	Mean EC50	pD$_2$OCT(±s.e.m)	Relative potency (−)-*p*-OCT	Fraction of (−)-*p*-OCT maximum	Isomeric activity ratio (−)/(+)	Relative potency (−)-NA
(−)-*p*-Octopamine	3	$5{\cdot}7\times10^{-7}$M	6·30(±0·15)	1·0	1·00	260	49
(−)-*p*-Synephrine	3	$8{\cdot}3\times10^{-8}$M	7·26(±0·26)	6·9	0·95	39	340
(−)-*m*-Octopamine	3	$8{\cdot}4\times10^{-4}$M	3·09(±0·09)	0·0007	0·52*	—	0·03
(−)-*m*-Synephrine	3	—	—	—	0·24	—	—
(−)-*o*-Octopamine	3	—	—	—	0·36*	—	—
(+)-*p*-Octopamine	3	$1{\cdot}5\times10^{-4}$M	3·98(±0·31)	0·004	0·67*	—	0·19
(+)-*p*-Synephrine	3	$3{\cdot}2\times10^{-6}$M	5·54(±0·15)	0·18	0·90	—	8·8
(+)-*m*-Octopamine	3	—	—	—	0·10*	—	—
(+)-*m*-Synephrine	3	—	—	—	0·00	—	—
(+)-*o*-Octopamine	3	—	—	—	0·00	—	—
(−)-Noradrenaline	3	$2{\cdot}8\times10^{-5}$M	4·63(±0·19)	0·02	0·75	—	1·0

*Satisfactory maximum not obtained (see Figs 1A, B). This is the mean of the responses to the highest concentration tested.

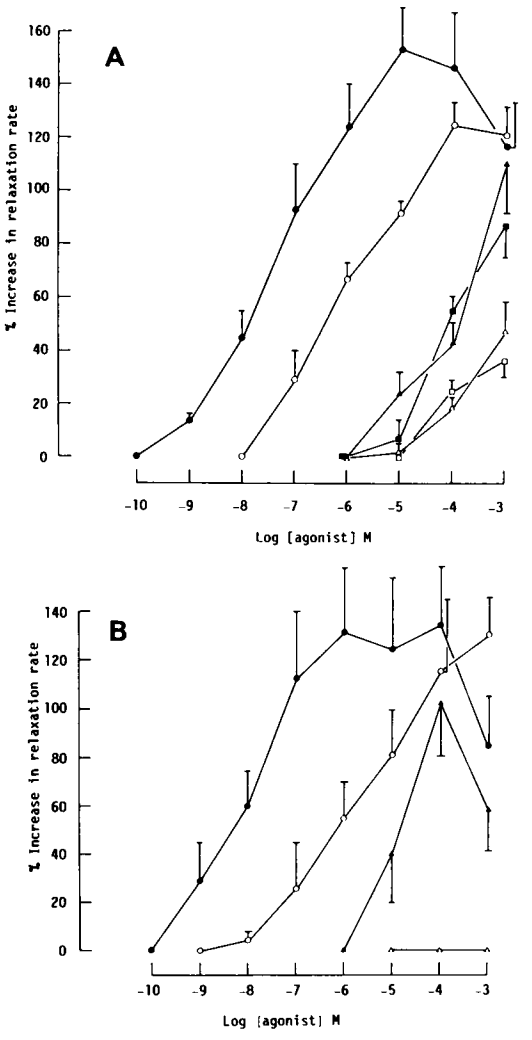

FIG. 2. The actions of the stereoisomers of octopamine (2A) and synephrine (2B) on the OCTOPAMINE$_{2B}$ receptors in the locust extensor-tibiae muscle. The results are plotted as the percentage increase in the rate of relaxation of SETi-induced twitch tension against Log agonist concentration (M) ●(−)-*p*, ○(+)-*p*-, ▲(−)-*m*, △(+)-*m*, ■(−)-*o*-, □(+)-*o*-isomers.

(−)-forms was *p*-synephrine > *p*-octopamine > *m*-synephrine > *m*-octopamine = *o*-octopamine. The activity of *p*-synephrine was almost six times that of *p*-octopamine whilst *m*-synephrine was an order of magnitude more potent than *p*-octopamine. In contrast, the rank order of potency of the (+)-forms was *p*-octopamine > *p*-synephrine > *m*-octopamine > *o*-octopamine, with the activity of *p*-octopamine being four times that of *p*-synephrine. The (+)-isomer of *m*-synephrine again failed to produce any increase in the relaxation rate of SETi-induced twitch tension when tested at concentrations up to 10^{-3}M. The EC50 values and average potency values for these compounds on OCTOPAMINE$_{2B}$ receptors are presented in Table 2 together with the isomeric activity ratios. The (−)-isomers were again the most active on the OCTOPAMINE$_{2B}$ receptors, with the (+)-isomers being one to two orders of magnitude weaker.

The isomeric forms which did not exhibit any agonist activity up to a concentration of 10^{-3}M in the above assays, were also investigated for possible antagonism by testing their ability to block the actions of a 30s pulse of 10^{-6}M (±)-octopamine on both the OCTOPAMINE$_{2A}$ and OCTOPAMINE$_{2B}$ receptors. However, (+)-*m*-synephrine did not show any antagonistic effects on the OCTOPAMINE$_{2A}$ or OCTOPAMINE$_{2B}$ receptors whilst (+)-*o*-octopamine did not show any antagonistic actions on the OCTOPAMINE$_{2A}$ receptors.

Effects on OCTOPAMINE$_1$ receptors slowing myogenic rhythm

The myogenic rhythm of contraction and relaxation found in a proximal bundle of muscle fibres in the locust extensor-tibiae muscle is reduced in frequency when OCTOPAMINE$_1$ receptors are activated (Evans 1981). The OCTOPAMINE$_1$ receptor activities of the (−)- and (+)-forms of *p*-, *m*- and *o*-octopamine are shown in Fig. 3A and of the (−)- and (+)-forms of *p*- and *m*-synephrine in Fig. 3B. The rank order of potency of the (−)-forms was *p*-synephrine > *p*-octopamine > *m*-synephrine > *m*-octopamine > *o*-octopamine. The activity of *p*-synephrine was almost four times that of *p*-octopamine whilst those of *m*-octopamine and *m*-synephrine

Table 2. The activity of octopamine and synephrine stereoisomers on OCTOPAMINE$_{2B}$ receptors in locust extensor-tibiae muscle.

	N	Mean EC50	pD$_2$OCT(±s.e.m)	Relative potency (−)-*p*-OCT	Fraction of (−)-*p*-OCT maximum	Isomeric activity ratio (−)/(+)	Relative potency (−)-NA
(−)-*p*-Octopamine	3	$9{\cdot}1\times10^{-8}$M	7·23(±0·33)	1·0	1·00	26	95
(−)-*p*-Synephrine	3	$1{\cdot}6\times10^{-8}$M	7·83(±0·13)	5·7	0·88	480	540
(−)-*m*-Octopamine	3	$3{\cdot}8\times10^{-4}$M	3·45(±0·14)	0·0002	0·72*	—	0·02
(−)-*m*-Synephrine	3	$4{\cdot}9\times10^{-5}$M	4·50(±0·30)	0·002	0·67	—	0·18
(−)-*o*-Octopamine	3	$7{\cdot}1\times10^{-4}$M	326(±0·24)	0·0001	0·56*	—	0·01
(+)-*p*-Octopamine	3	$2{\cdot}4\times10^{-6}$M	5·74(±0·21)	0·04	0·81	—	3·6
(+)-*p*-Synephrine	3	$7{\cdot}7\times10^{-6}$M	5·12(±0·68)	0·01	0·84	—	1·1
(+)-*m*-Octopamine	3	—	—	—	0·31*	—	—
(+)-*m*-Synephrine	3	—	—	—	0·00	—	—
(+)-*o*-Octopamine	3	—	—	—	0·24*	—	—
(−)-Noradrenaline	3	$8{\cdot}6\times10^{-6}$M	5·07(±0·15)	0·01	1·00	—	1·0

* Satisfactory maximum not obtained (see Figs. 2A, B). This is a mean of the responses to the highest concentration tested.

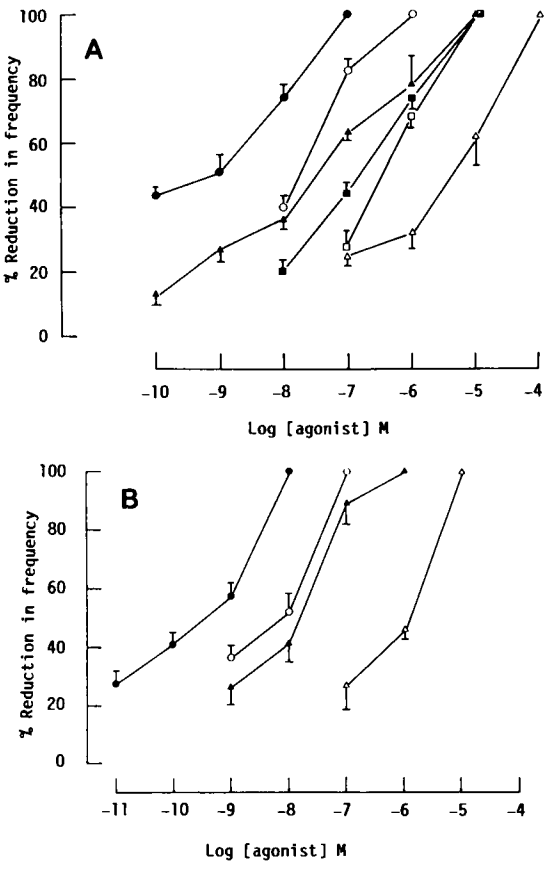

FIG. 3. The actions of the stereoisomers of octopamine (3A) and synephrine (3B) on the OCTOPAMINE$_1$ receptors in the locust extensor-tibiae muscle. The results are plotted as the percentage reduction in frequency of the myogenic rhythm in the muscle against Log agonist concentration (M) ●(−)-*p*, ○(+)-*p*-, ▲(−)-*m*, △(+)-*m*, ■(−)-*o*-, □(+)-*o*-isomers.

were about 20-fold less and that of *o*-octopamine about 125-fold less. The rank order of potency of the (+)-forms was *p*-synephrine > *p*-octopamine > *o*-octopamine > *m*-synephrine > *m*-octopamine. All the (−)- and (+)- isomers tested in this assay were able to inhibit the myogenic rhythm completely. The EC50 values and mean potency values for those compounds on OCTOPAMINE$_1$ receptors are presented in Table 3 together with their isomeric activity ratios.

In all cases the (−) isomers were more potent with the (−)-*para*-forms being 16–33 fold more potent than the (+)-*para*-forms, the (−)-*meta*-forms being 70–200 fold more potent than the (+)-*meta*-forms and (−)-*o*-octopamine being twice as potent as (+)-*o*-octopamine.

Discussion

The different octopamine receptor subtypes on the extensor-tibiae muscle of the locust hindleg show differences in the rank order of potency of the (+)- and (−)-enantiomers of the structural isomers of octopamine and synephrine. In general the *para*-isomers were always more potent than the *meta*- or *ortho*-isomers. In addition *p*-synephrine was from 3–45 fold more potent than *p*-octopamine for all the receptor subtypes and stereoisomeric forms tested (except for the action of the (+)-isomers on the OCTOPAMINE$_{2B}$ receptors where octopamine was four times more potent than synephrine). At the OCTOPAMINE$_{2A}$ receptors both the (+)- and (−)-forms of *m*-octopamine were more potent than the corresponding (+)- and (−)-forms of *m*-synephrine, whilst the converse was true for OCTOPAMINE$_1$ receptors. At OCTOPAMINE$_{2B}$ receptors (+)-*m*-octopamine was more potent than (+)-*m*-synephrine, whilst (−)-*m*-synephrine was more potent than (−)-*m*-octopamine. The *ortho*-forms of octopamine were only weak agonists of the OCTOPAMINE$_2$ receptor subtypes but were much more potent agonists of the OCTOPAMINE$_1$ receptors with EC50 values around 10^{-7}M. In general all the isomeric forms of octopamine and synephrine tested were much more potent agonists of OCTOPAMINE$_1$ receptors than OCTOPAMINE$_2$ receptors as judged by their EC50 values, their average potency values and by their threshold responses. This was particularly noticeable for the *meta*-isomers, where (−)-*m*-octopamine, for example, was 32000 times more potent on OCTOPAMINE$_1$ than OCTOPAMINE$_{2A}$ receptors and 15000 times more potent on OCTOPAMINE$_1$ than OCTOPAMINE$_{2B}$ receptors.

In all cases the (−)-stereoisomeric forms of all the structural isomers tested were more potent than the (+)-forms. This agrees well with previous studies on the octopamine receptors in this preparation (Evans 1981, 1984a) and in other insect preparations (Harmar & Horn 1977; Roberts & Walker 1981). The isomeric activity ratios varied widely

Table 3. The activity of octopamine and synephrine stereoisomers on OCTOPAMINE$_1$ receptors in locust extensor-tibiae muscle.

	N	Mean EC50	pD$_2$OCT(±s.e.m)	Relative potency (−)-*p*-OCT	Isomeric activity ratio (−)/(+)	Relative potency (−)-NA
(−)-*p*-Octopamine	3	$1{\cdot}1\times10^{-9}$ M	9·12(±0·33)	1·0	16	210
(−)-*p*-Synephrine	3	$3{\cdot}0\times10^{-10}$M	9·72(±0·28)	3·7	33	770
(−)-*m*-Octopamine	3	$2{\cdot}6\times10^{-8}$ M	7·61(±0·12)	0·04	210	8·8
(−)-*m*-Synephrine	3	$1{\cdot}6\times10^{-8}$ M	7·81(±0·09)	0·07	69	14
(−)-*o*-Octopamine	3	$1{\cdot}4\times10^{-7}$ M	6·90(±0·14)	0·008	2·4	1·6
(+)-*p*-Octopamine	3	$1{\cdot}8\times10^{-8}$ M	7·76(±0·09)	0·06	—	13
(+)-*p*-Synephrine	3	$1{\cdot}1\times10^{-8}$ M	8·08(±0·23)	0·10	—	21
(+)-*m*-Octopamine	3	$5{\cdot}4\times10^{-6}$ M	5·35(±0·19)	0·0002	—	0·04
(+)-*m*-Synephrine	3	$1{\cdot}1\times10^{-6}$ M	5·96(±0·04)	0·001	—	0·21
(+)-*o*-Octopamine	3	$3{\cdot}4\times10^{-7}$ M	6·46(±0·07)	0·003	—	0·68
(−)-Noradrenaline	3	$2{\cdot}3\times10^{-7}$ M·	6·70(±0·13)	0·005	—	1·0

for the different isomers tested, ranging from a factor of 2 for *o*-octopamine on the $OCTOPAMINE_1$ receptors to a value of 480 for *p*-synephrine on the $OCTOPAMINE_{2B}$ receptors. There is evidence to suggest that the naturally occurring enantiomer of *p*-octopamine in insects is the (−)-form (Goosey & Candy 1980; Starratt & Bodnaryk 1981) but the abolute configuration of this isomer (assumed for many years to be D(*R*)) has not been determined by direct experimentation.

A major finding of the present study is that the isomeric forms of octopamine and synephrine differ in their rank order of potency on insect octopamine receptors from that found for α- and β-adrenoceptor subtypes in vertebrates (see Jordan et al 1987; Brown et al 1988). On vertebrate adrenergic receptor subtypes, *meta*-isomers are in general more potent than the corresponding *para*-isomers whereas the converse is always true of insect octopamine receptors. The only exception to this is the action of the (−)-forms of octopamine on B_1 receptors, where *m*-octopamine and *p*-octopamine are equipotent. Insect octopamine receptors are also different from vertebrate adrenoceptors in that (−)-*p*-octopamine and (−)-*p*-synephrine are much more potent agonists than noradrenaline at the former (see Tables 1–3), whereas the converse is true at the latter (see Evans 1981, 1984a). A detailed description of the pharmacological properties of insect octopamine receptors is given by Evans (1981, 1984a, 1987). The results of the present study confirm the important principle that a series of enantiomers can be used to distinguish pharmacologically between closely related receptors (Jordan, et al 1987; Brown et al 1988).

Previous studies on the actions of the different stereoisomeric forms of the structural isomers of octopamine and synephrine on vertebrate α- and β-adrenoceptor subtypes (Jordan et al 1987; Brown et al 1988) have concluded that *m*- and *p*-octopamine are unlikely to have any physiological functions that are mediated by any one of the four adrenoceptor subtypes. The present paper emphasizes the pharmacological differences between insect octopaminergic receptors and vertebrate adrenergic receptors and suggests that the rank order of potency of the different stereoisomeric forms of the structural isomers of octopamine and synephrine may be useful in the differentiation of specific octopaminergic receptors from adrenergic receptors in the vertebrate nervous system. Such a distinction is essential for a better understanding of the functional role of octopamine in vertebrates.

Acknowledgements

This work was supported by an AFRC University Link Award to P.D.E. and J.M.M.

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