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A contribution, from a possible local anaesthetic action, to the effects of yohimbine on evoked noradrenaline overflow

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Both yohimbine (0·1 to 10 μM) and phentolamine (10 μM) increased the tritium overflow evoked by electrical stimulation (2·5 Hz, 1 ms, 15 V, for 90 s every 20 min) of mouse isolated vas deferens previously incubated with (−)-[^{3}H]noradrenaline. At their maximally effective concentrations, phentolamine (10 μM) produced an effect that was sustained over the 2 h of the experiment while the effect of yohimbine (6 μM) decreased by about 60% over the first 40 min and was then sustained at a lower level. At higher concentrations of yohimbine, the increase in evoked tritium overflow was less marked and at the highest concentration tested (30 μM) evoked overflow was reduced below the levels seen before exposure to the drug. It is concluded that at concentrations maximally effective in inhibiting the presynaptic α-adrenoceptor-mediated mechanism controlling transmitter release, the known local anaesthetic effect of yohimbine may contribute to the overall effect on evoked transmitter overflow.

Yohimbine has been used extensively in the investigation of α-adrenoceptor involvement in the control of transmitter release, but it possesses other actions in addition to its ability to block α_2-adrenoceptors. For example, α_1-adrenoceptors are also blocked and the concentration of yohimbine required is only about 5-fold greater than that required to block α_2-adrenoceptors (McGrath 1982). Dopamine receptor blockade has been reported at yohimbine concentrations of about 1 μM (Scatton et al 1980) and 5-hydroxytryptamine receptors may be affected at slightly lower concentrations (Reid & Rand 1952; Shaw & Woolley 1953; Sanghvi & Gershon 1974; Matthews & Smith 1980). At higher concentrations, muscarinic receptors may be blocked and cholinesterase inhibited (Lambert et al 1978). A local anaesthetic or membrane stabilizing action has been reported in squid axons (Lipicky et al 1977), frog Ranvier node membrane (Revenko et al 1982) and in myocardial cells (Azuma et al 1978) and concentrations between 20 and 100 μM are generally required to show this effect. However, the results reported herein indicate that at concentrations that could be used to block α-adrenoceptors, a possible local anaesthetic action of yohimbine may contribute to the overall effect on evoked transmitter overflow.

Method

Male mice (T.O. strain; 20–30 g) were killed by a blow on the head; one vas deferens was removed and cleared of attached mesentery. Ligatures were tied to each end and the tissue was incubated at 37 °C for 45 min in physiological saline (NaCl 118, KCl 4·75, $CaCl_2$ 2·54, KH_2PO_4 0·93, $NaHCO_3$ 25, glucose 11 mM also containing ascorbic acid 11, disodium ethylenediamine tetra-acetic acid 27 and 17-β-oestradiol 3·7 μM, gassed with 5% carbon dioxide in oxygen) containing 0·071 μM (−)-[^{3}H]noradrenaline (14 Ci mmol^{-1}). The tissue was then mounted between two parallel platinum wire electrodes and placed in a tissue bath (1·0 ml) which was drained and refilled with physiological saline every 2 min. After being washed for 35 min the tissue was stimulated electrically (rectilinear pulses, 2·5 Hz, 1 ms duration, 15 V 150–220 mA for 90 s) every 20 min; under these conditions the evoked overflow of tritium is entirely calcium-dependent and is abolished by cinchocaine (3 μM). The effluent from the tissue bath was collected in scintillation vials, liquid scintillation cocktail was added (10 ml; Fisons 'FisoFluor') and the vials were counted for tritium in a Packard 360C liquid scintillation counter. Correction for quench was applied using the spectral index of the external standard.

Drugs were applied to the tissue though the bulk of the physiological saline and allowed to come into contact with the tissue 14 min before the next stimulation period. At the end of the experiment the tissue was solubilized in 1·0 ml of Fisons 'FisoSolve', mixed with 0·25 ml glacial acetic acid, 0·25 ml water and 10 ml FisoFluor and counted for tritium as above. Resting tritium overflow was taken as the average of the tritium in the two samples collected immediately before a stimulation (S) period. Evoked tritium overflow was taken as that appearing in the effluent in excess of that expected from the resting overflow. Both resting and evoked tritium overflows were expressed in fractional terms by dividing these values by the tissue tritium content at the time the resting samples were collected or at the time of stimulation as appropriate.

Treatment of results. For each tissue a ratio has been calculated for both the fractional resting and the fractional evoked overflows occurring in the later stimulation period(s) to the values for these parameters found in the first stimulation period for which samples were collected (Sx/S2). This ratio has been used to quantitate the effects of drugs. Means and standard errors (m ± s.e.m.) have been calculated where appropriate and the number of values contributing to each mean is shown (n). Tests for statistical significance utilized Student's *t*-test unless otherwise stated.

Drugs used. Ascorbic acid (BDH), ethylenediamine tetra-acetic acid (BDH), phentolamine mesylate

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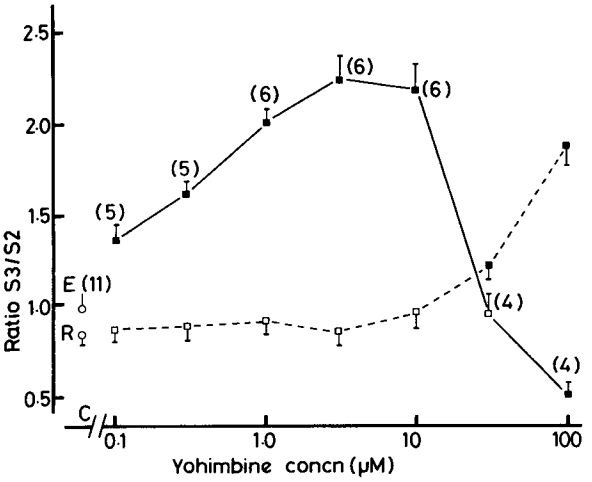

FIG. 1. Ratio (S3/S2) of the fractional resting (R; - - - -) and of the fractional evoked (E; ——; 2·5 Hz, 1 ms, 15 V, 150–220 mA for 90 s) tritium overflow from mouse vas deferens previously incubated with (−)-[^{3}H]noradrenaline to the corresponding values found in an initial control period (S2) after which tissues were exposed to various concentrations of yohimbine. Points represent means and the number of observations contributing to each point is shown in parentheses. The bars show the standard error and the solid points represent those significantly different statistically ($P < 0.05$; Student's t-test) from the corresponding values in control tissues exposed to physiological saline alone (C; ○).

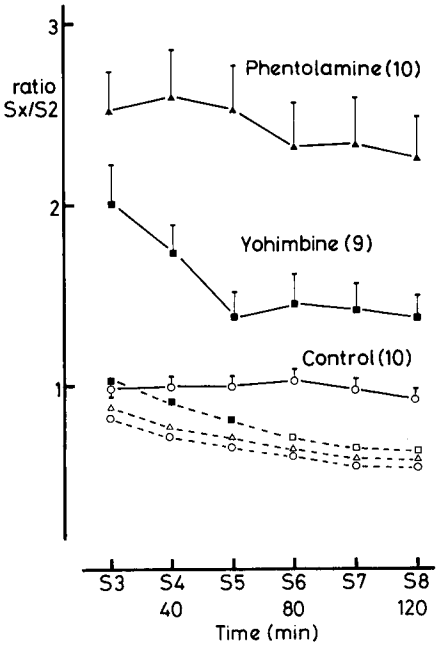

FIG. 2. Ratio (Sx/S2) of the fractional resting (- - - -) and of the fractional evoked (——; 2·5 Hz, 1 ms, 15 V, 150–220 mA for 90 s) tritium overflow from mouse vas deferens previously incubated with (−)-[^{3}H]noradrenaline to the corresponding values found in an initial control period (S2) after which tissues were either exposed to phentolamine (△, 10 μM), to yohimbine (□, 6 μM) or to neither drug (○, control). Periods of stimulation (S2 to S8) were applied at 20 min intervals. The points represent means and the number of observations contributing to each point is shown in parentheses. Standard errors are shown for the data on evoked overflow but have been omitted from the points showing resting overflow for clarity. Solid points represent those significantly different statistically ($P < 0.05$; Student's t-test) from the corresponding values in control tissues.

(Ciba), 17-β-oestradiol (Sigma), yohimbine hydrochloride (Sigma). The radiolabelled materials were obtained from the Radiochemical Centre, Amersham.

Results

The first period of stimulation (S1) evoked an overflow of tritium which was not always representative of that occurring in later periods of stimulation and data obtained from this period were discarded. At the start of the second period of stimulation (S2) the tissues contained large amounts of tritium ($2.23 \pm 0.09 \times 10^6$ d min^{-1}; m ± s.e.m.; n = 66) and there was no statistically significant difference between the mean tritium content of the groups of tissues ($P > 0.3$). The resting tritium overflow before the second stimulation period (S2) averaged 5526 ± 191 d min^{-1} (m ± s.e.m.; n = 66) and the overflow evoked by electrical stimulation averaged 15607 ± 948 d min^{-1} (m ± s.e.m.; n = 66). The corresponding figures in fractional terms were $2.72 \pm 0.15 \times 10^{-3}$ and $6.99 \pm 0.30 \times 10^{-3}$ respectively.

Exposure of the tissues to yohimbine 14 min before S2, produced little change in fractional resting tritium overflow at S2 except when the drug was at the highest concentrations, where a statistically significant ($P < 0.001$) increase was produced. Fractional evoked tritium overflow was increased in a concentration-dependent manner, a maximal increase of about 2-fold being produced at about 3 μM. Concentrations above 10 μM produced a precipitate fall in evoked overflow (Fig. 1).

In control experiments involving a greater number of stimulation periods, fractional resting tritium overflow declined as the experiment progressed. Exposure of the tissues to phentolamine (10 μM) did not alter fractional resting tritium overflow ($P > 0.4$) or the rate of decline. Exposure to yohimbine (6 μM) produced a small (about 16%) but statistically significant increase in the fractional resting tritium overflow ($P < 0.001$) but did not markedly alter the rate of decline (Fig. 2).

In untreated tissues, fractional-evoked tritium overflow declined slightly towards the end of the experimental period (2 h) though a paired t-test showed that the decline was not significant statistically ($P > 0.6$) (Fig. 2). Phentolamine (10 μM) produced an increase in fractional-evoked tritium overflow of about 2·5-fold

which was largely maintained during the remainder of the exposure to phentolamine and a paired *t*-test showed that the fractional-evoked overflows in periods S3 or S4 were not significantly different statistically from that in S8 ($P > 0{\cdot}6$ in both cases). Yohimbine (6 μM) increased fractional-evoked tritium overflow to a slightly smaller extent (2-fold) and the effect declined sharply by about 60% during the first hour of exposure though even after 2 h exposure the fractional-evoked tritium overflow was still significantly greater than that in the untreated control tissues ($P < 0{\cdot}01$) (Fig. 2).

Discussion

Tritium overflow evoked by electrical stimulation of a tissue previously incubated with tritiated noradrenaline represents the difference between the tritium released from the tissue and that taken back up into the cells. The tritium is not all present as noradrenaline but will exist, in part, in metabolites formed from the transmitter after release, or released in conjunction with the transmitter. No attempt has been made to separate the tritium into identified molecular species and it must be remembered throughout this work that changes in tritium overflow may not accurately reflect changes in transmitter concentrations in the synaptic cleft (Marshall 1983).

Although there is considerable controversy about the nature of the motor transmitter in the mouse vas deferens (Ambache & Zar 1971; Jones & Spriggs 1975; Jenkins et al 1976) it is generally agreed by most workers that this tissue does contain noradrenergic nerves which will release noradrenaline on stimulation. This evoked release of noradrenaline is under the control of presynaptic α-adrenoceptors (Marshall 1983) and it is not surprising therefore that both α-adrenoceptor blocking agents produced an increase in evoked tritium overflow. The concentration of both agents was chosen so that a just maximal effect on fractional-evoked tritium overflow was produced (see Hughes 1978 for data on phentolamine). The effect of phentolamine was well maintained indicating that the nerve can sustain transmitter output even when demand is high. Yohimbine also increased evoked tritium overflow though the effect was not well maintained. This cannot be due to exhaustion of tritium or noradrenaline supplies, since the effect was smaller than that produced by phentolamine where the increase in tritium overflow is maintained. Furthermore, the decline in effect ceases after about 40 min and thereafter evoked tritium overflow is maintained at a lower level. Although no direct evidence of local anaesthesia is reported here, of the known actions of yohimbine, it seems most likely that the local anaesthetic properties could account for these observations.

A local anaesthetic effect may account for the slightly smaller maximal effect of yohimbine on fractional evoked tritium overflow than was obtained with phentolamine. It would be expected that two 'specific' α_2-adrenoceptor blockers would produce equal increases in fractional-evoked tritium overflow providing a sufficient concentration was used. It would also explain the steep fall in fractional-evoked tritium overflow at the highest concentrations where the local anaesthetic effect would predominate over the α_2-adrenoceptor blocking actions seen at lower concentrations.

This local anaesthetic effect may account for the bell-shaped concentration response relationship for the effect of yohimbine on evoked noradrenaline overflow from rabbit pulmonary artery (Rhodes et al 1983), its inhibitory effect on evoked noradrenaline (tritium) overflow in radial artery (Kalsner & Chan 1979) and for the ability of yohimbine to inhibit the electrically-evoked release of GABA from cerebral cortex slices (Arbilla et al 1982). It would also explain why Drew (1977) found that yohimbine inhibited the electrically-evoked twitch response of the rat vas deferens while similar concentrations of phentolamine, piperoxan and thymoxamine enhanced the response.

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