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Yohimbine affects the evoked overflow of neurotransmitters from rat brain slices by more than one mechanism

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Both yohimbine (0·1–10 μM) and phentolamine (10 μM) increased the tritium overflow evoked by electrical stimulation (3Hz, 2 ms, 18 mA for 120s every 20 min) of rat brain cortex slices previously incubated with [^{3}H](−)-noradrenaline. At their maximally effective concentrations, neither of these compounds produced an effect which was fully maintained over the 1 h of the experiment, but the decline in effect of yohimbine (1·25 μM) was more marked, falling sharply to reach, after 1 h, 25% of the effect observed after 20 min, whereas phentolamine only declined to 60% of the effect after 20 min. In rat brain cortex slices previously incubated with [^{3}H]5-hydroxytryptamine ([^{3}H]5-HT), the tritium overflow evoked by electrical stimulation (3 Hz, 2 ms, 40 mA for 120 s) was increased by yohimbine (0·1–1 μM) and phentolamine (0·1–10 μM), but a higher concentration of yohimbine (10 μM) decreased evoked overflow below the levels seen in the absence of either drug. It is concluded that, at concentrations effective in inhibiting the presynaptic α-adrenoceptor-mediated mechanisms controlling transmitter release in rat brain slices, a non-α-adrenoceptor-mediated, possible local anaesthetic, action of yohimbine contributes to the overall effect of this drug on transmitter overflow.

Yohimbine, a relatively selective α_2-adrenoceptor blocking agent, has been used extensively in the investigation of α-adrenoceptor-mediated feedback mechanisms regulating transmitter release, both in peripheral tissues (see Langer 1981) and in the CNS; for example, Ennis (1983). However, yohimbine also possesses a number of other pharmacological actions, including the ability to decrease the excitability of neuronal membranes (for references, see Goodall et al 1984). Recently, we have suggested that this local anaesthetic action may contribute to the overall effect of yohimbine on evoked noradrenaline overflow from mouse isolated vas deferens (Goodall et al 1984). In the present study we have investigated the effects of yohimbine on tritium overflow from rat brain occipital cortex slices previously incubated with [^{3}H](−)-noradrenaline or [^{3}H]5-HT and conclude that, in central neurons also, yohimbine exerts a slowly developing inhibitory effect on transmitter release, which is not restricted only to noradrenergic neurons and can be explained by the known local anaesthetic action of this drug.

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METHODS

Male Wistar rats (125–175 g) were decapitated and the brain rapidly removed and immersed in cold, aerated (5% CO_2, 95% O_2), physiological saline (NaCl 118, KCl 4·75, $CaCl_2$ 1·30, $MgSO_4$ 1·2, $NaHCO_3$ 25·0, KH_2PO_4 1·2, glucose 1·0, disodium ethylenediaminetetraacetic acid 0·03, ascorbic acid 0·06 mM, and, in experiments involving [^{3}H](−)-noradrenaline, 17β-oestradiol 4 μM). Occipital cortical tissue was removed and cut into slices (5·0 × 2·0 × 0·4 mm) by means of a McIlwain tissue chopper kept at 4 °C.

Eight slices were incubated at 37 °C for 45 min in 2 ml of physiological saline containing 0·1 μM [^{3}H](−)-noradrenaline or 0·1 μM [^{3}H]5-HT, were briefly washed, and placed in a superfusion chamber (volume 0·35 ml) between two platinum ring electrodes. The slices were then superfused with saline solution kept at 37 °C, at a rate of 0·5 ml min^{-1}. After an initial 30 min wash period, the superfusate containing the tritium overflowing from the brain slice was collected in sequential 4 min samples until the end of the experiment. Each slice was subjected to 2 min periods of electrical stimulation [S1 to Sx; rectilinear pulses, 3 Hz, 2 ms pulse width, 18 mA (experiments with [^{3}H](−)-noradrenaline), 40 mA

(experiments with [^{3}H]5-HT)] applied as required, at 20 min intervals.

To determine effects on resting and evoked tritium overflow, drugs under test were added to the superfusion medium in the collection period following the previous stimulation period. These drugs then generally remained in contact with the tissue until the end of the experiment, when each slice was solubilized in 1 ml of tissue solubilizer (Fisosolve, Fisons) and the tritium in the superfusate and tissue samples determined by liquid scintillation counting after addition of 18 ml Fisofluor 1 scintillation cocktail (Fisons).

The tritium content of the sample collected immediately before a period of stimulation was taken as the resting overflow and was divided by the tritium content of the tissue in that period to give the fractional resting overflow. The tritium overflow appearing in the sample in which electrical stimulation was applied and in subsequent samples in which it was in excess of that expected from the resting overflow, was summed to give the evoked overflow and this was divided by the tissue content of tritium at the onset of stimulation to give the fractional evoked overflow.

Treatment of results

For each tissue a ratio has been calculated for both the fractional resting and fractional evoked overflows occurring in the later stimulation period(s) to the values for these parameters found in the first stimulation period (Sx/S1). This ratio has been used to quantify the effects of drugs. Mean and standard error of the mean (m ± s.e.m.) have been calculated where appropriate and the number of values contributing to each mean is shown (n). For tests for statistical significance, Student's *t*-test was used unless otherwise stated.

Drugs used

Ascorbic acid (BDH), disodium ethylenediaminetetraacetic acid (BDH), phentolamine mesylate (Ciba) 17-β-oestradiol (Sigma), yohimbine hydrochloride (Sigma). The radiolabelled materials: [^{3}H](−)-noradrenaline (14 Ci mmol^{-1}) and [^{3}H]-5-HT (13 Ci mmol^{-1}) were obtained from the Radiochemical Centre, Amersham.

RESULTS

Tissues incubated with [^{3}H](−)-noradrenaline

At the start of the first period of stimulation the tissues contained large amounts of tritium (2·00 ± 0·08 × 10^5 d min^{-1}; n = 36) and there was no statistically significant difference between the mean tritium content of the appropriate control group and any of the other groups of tissues ($P > 0·1$). The resting tritium overflow before the first stimulation period averaged 2084 ± 61 d min^{-1} and the overflow evoked by electrical stimulation averaged 3851 ± 266 d min^{-1} (n = 36). Expressed in fractional terms the corresponding figures were 1·06 ± 0·03 × 10^{-2} and 1·95 ± 0·12 × 10^{-2}, respectively.

In control tissues fractional resting overflow decreased as the experiment progressed but fractional evoked overflow remained relatively constant, there being no statistically significant difference between the ratio of fractional evoked overflow at S2 (i.e. S2/S1) and that at S4 (i.e. S4/S1) ($P > 0·7$; paired *t*-test). Under these experimental conditions the overflow of tritium evoked by electrical stimulation was entirely dependent on the presence of calcium ions in the superfusion medium, and was abolished by tetrodotoxin (0·31 μM) and by cinchocaine (26·3 μM).

Exposure of the tissues to various concentrations of yohimbine for 16 min before the second stimulation period (S2) produced a concentration-dependent increase in the fractional overflow of tritium evoked by the second period of stimulation (Fig. 1). The minimal effective concentration was

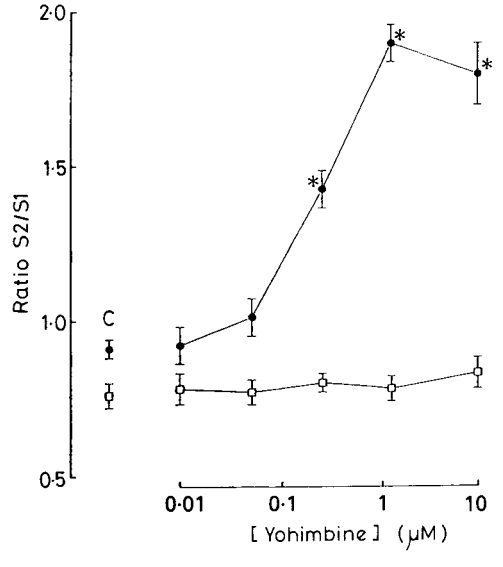

FIG. 1. Effect of yohimbine on the fractional resting (□) and evoked (●) overflow of tritium from rat occipital cortex slices previously incubated with [^{3}H](−)-noradrenaline. Yohimbine was applied to the superfusion fluid after S1 and remained in contact with the tissues for the remainder of the experiment. Each point represents the mean ± s.e.m. of 3–10 determinations. C—control tissues exposed to physiological saline alone. *—Values significantly different ($P < 0·05$) from corresponding control values.

about 50 nM and a maximal effect was seen at about 1·25 μM when the fractional evoked tritium overflow almost doubled. Fractional resting tritium overflow was not affected significantly ($P > 0·2$) at any of the concentrations of yohimbine used.

Prolonged exposure of tissues to yohimbine (1·25 μM) showed that the increase in fractional evoked tritium overflow was not fully maintained but declined sharply to reach, at the fourth stimulation period, about 25% of the original effect (Fig. 2). Tissues first exposed to yohimbine 16 min before the fourth stimulation period (S4) also showed an increase in fractional evoked tritium overflow which reached a value higher than that found at S4 in tissues which had been exposed to yohimbine before S2, the value for the ratio at these two periods being 1·57 ± 0·13 and 1·22 ± 0·07, respectively (n = 6; $P <$ 0·05). The increase was significantly smaller, however ($P < 0·02$), than that produced by yohimbine at S2 when applied 16 min earlier where the ratio was 2·11 ± 0·12 (n = 6). In comparison with control tissues yohimbine did not affect resting tritium overflow whether applied before S2 or before S4 ($P > 0·3$).

Phentolamine (10 μM) more than doubled the fractional evoked tritium overflow at S2 when applied 16 min previously (Fig. 2). The effect was not fully maintained and declined to reach, at S4, about 60% of the original response, a less sharp decline than seen with yohimbine which declined to about 25% of the original response. When applied 16 min before S4, phentolamine also increased fractional evoked tritium overflow and the ratio increased to 1·99 ± 0·17 (n = 6), a value not significantly different statistically ($P > 0·4$) from that obtained at S4 in tissues exposed to phentolamine from before S2 where the ratio was 1·81 ± 0·19 (n = 6).

Phentolamine produced a statistically significant increase ($P < 0·05$) in fractional resting overflow whether applied before S2 or before S4 though in both cases the size of the effect was small (less than 10%).

Tissues incubated with [³H]5-hydroxytryptamine

At the start of the collection period, immediately before the first period of stimulation (S1), the tissues contained large amounts of tritium (7·29 ± 0·18 × 10^4 d min^{-1}; n = 53) and there was no statistically significant difference between the mean tritium content of the appropriate control group and any of the other groups of tissues ($P > 0·2$). The resting tritium overflow before the first stimulation period averaged 3259 ± 79 d min^{-1} and the overflow

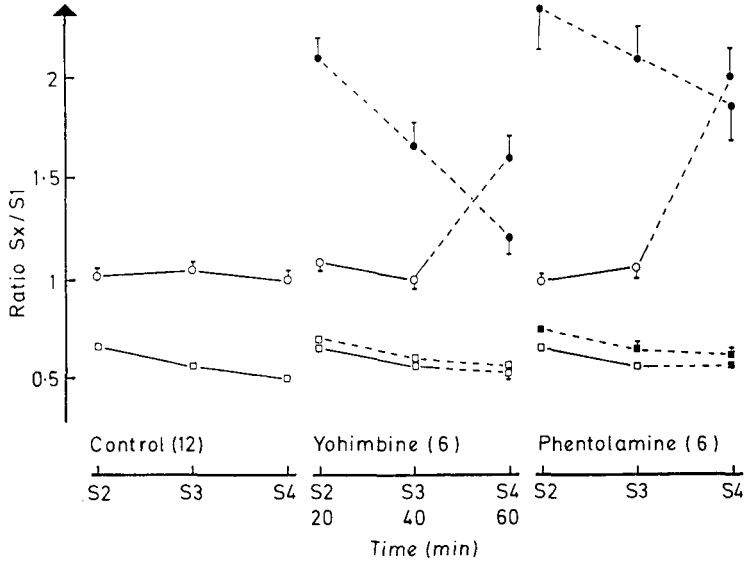

FIG. 2. Ratio of the fractional resting (□) or of the fractional evoked (○) tritium overflow from rat occipital cortex slices previously incubated with [³H](−)-noradrenaline to the corresponding values found in an initial control period of stimulation (S1). Tissues were superfused with physiological saline alone (—) or with physiological saline containing either yohimbine (1·25 μM) or phentolamine (10 μM) as indicated by the broken lines (- - -). Periods of stimulation (S1 to S4) were applied at 20 min intervals. Points represent means of 6 (drug treated) or 12 (control) tissues. Standard errors of the means are shown but for some of the data on resting overflow these were within the area occupied by the point. Solid points represent those significantly different ($P < 0·05$) from the corresponding values in tissues exposed to physiological saline alone (control).

evoked by electrical stimulation averaged 1236 ± 113 d min^{-1} (n = 53). Expressed in fractional terms the corresponding figures were 4·49 ± 0·12 × 10^{-2} and 1·89 ± 0·16 × 10^{-2}, respectively. Under these experimental conditions the tritium overflow evoked by electrical stimulation was entirely dependent on the presence of calcium ions in the superfusion medium and was abolished by tetrodotoxin (0·31 μM) and cinchocaine (26·3 μM).

As can be seen from Fig. 3, yohimbine had no effect on fractional resting tritium overflow even at the highest concentration tested, but did increase fractional evoked tritium overflow at both 0·1 μM ($P < 0·001$) and 1 μM ($P < 0·05$), though the effect was not large. At 10 μM, fractional evoked tritium overflow was reduced in comparison with control tissues ($P < 0·01$). In contrast, phentolamine (0·1–10 μM) increased fractional evoked tritium overflow in a concentration-dependent manner, producing an approximate increase of 58% at 10 μM.

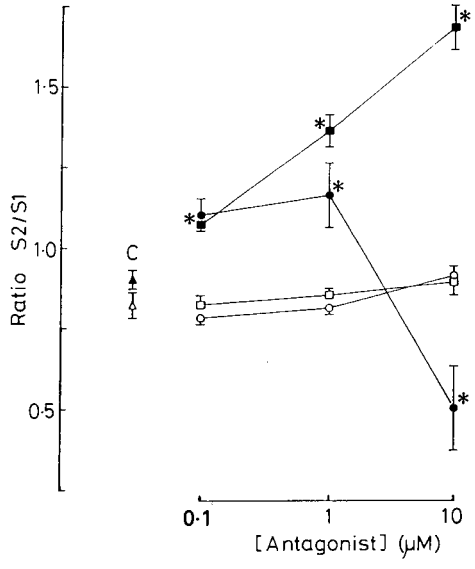

FIG. 3. Effect of yohimbine (circles) and phentolamine (squares) on the fractional resting (open symbols) and evoked (solid symbols) overflow of tritium from rat occipital cortex slices previously incubated with [^{3}H]5-HT. The antagonists were applied to the superfusion fluid after S1 and remained in contact with the tissues for the remainder of the experiment. Each point represents the mean ± s.e.m. of 6–12 determinations. C—Control tissues exposed to physiological saline alone. *—Values significantly different ($P < 0·05$) from corresponding values exposed to physiological saline alone (shown as triangles).

In a single experiment using a more prolonged exposure to yohimbine (5 μM), a progressive decline in fractional evoked tritium overflow was observed over at least the first 36 min of exposure and the ratio of fractional evoked tritium overflow at S2, S3, S4 and S5 to that at S1 was 0·59, 0·26, 0·21 and 0·19, respectively, while the corresponding ratios in a control tissue were 1·03, 1·06, 0·97 and 0·90.

DISCUSSION

Tritium overflow evoked by electrical stimulation of a tissue previously incubated with tritiated noradrenaline or 5-hydroxytryptamine 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 the appropriate transmitter 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).

Electrical stimulation of cortical slices, previously incubated with [^{3}H](−)-noradrenaline, evoked a fractional overflow per pulse of 5·42 × 10^{-5}, which is comparable with values of 9·47 × 10^{-5} (Pelayo et al 1980), 8·11 × 10^{-5} (Gross & Schumann 1981) and 5·75 × 10^{-5} (Starke & Montel 1977), though differences in conditions make such comparisons of limited value.

In cortical slices previously incubated with [^{3}H](−)-noradrenaline, both phentolamine and yohimbine increased fractional evoked tritium overflow. This is compatible with the presence of a functional α-adrenoceptor-mediated feed-back control regulating transmitter release from noradrenergic neurons in cortical slices as has been shown by other workers (see Pelayo et al 1980 for references). The effects of yohimbine and of phentolamine were not maintained during prolonged exposure, the decline being more marked with yohimbine. A number of factors could contribute to this decline. Firstly, the effect could be related to the amount of tritium released by the electrical stimuli employed, since it is only apparent when evoked release is greater than that found in untreated tissues. The total loss of tritium up to stimulation period S4 in experiments where phentolamine was present throughout was approximately 30% of total tissue tritium, whilst in control experiments the tritium loss was approximately 25%. This small difference in available tritium is unlikely to account completely for the lack of maintenance of the effect of phentol-

amine. Secondly, a deterioration in the functional capacity of the slice may be occurring. If the increase in fractional evoked tritium overflow produced by phentolamine or yohimbine is taken as a measure of the operation of the α-adrenoceptor-mediated feedback mechanism, then a smaller feedback on release appears to be occurring at S4 compared with S2. The constancy of the fractional evoked overflow in control tissues does not weigh against this possibility as it may be that constancy is achieved by a progressive withdrawal of feedback inhibition, compensating for the deterioration in the slice. However, if these were the only factors involved it would be expected that the effect of phentolamine and yohimbine would decline in parallel. Since this is not the case, an additional component must be present with yohimbine. The time course of the decline in effect of yohimbine is compatible with a slowly developing local anaesthetic action. Thus, exposure to yohimbine before S4 produced a fractional tritium overflow at S4 which was significantly greater than that seen at S4 in slices exposed to yohimbine before S2 (an extra 40 min contact time). With phentolamine, exposure before S4 produced a fractional tritium overflow which was not significantly different from that seen at S4 in tissues exposed to phentolamine before S2. This indicates that, for phentolamine, passage of time alone, not duration of exposure to the drug, is responsible for the decline in drug effect. These results are therefore compatible with experiments in mouse vas deferens previously incubated with [^{3}H](−)-noradrenaline (Goodall et al 1984), where the increase in evoked tritium overflow produced by phentolamine, but not that by yohimbine, is maintained over 120 min (six stimulation periods).

In cortical slices previously incubated with [^{3}H]-5-HT, electrical stimulation evoked a similar fractional overflow per pulse ($5{\cdot}25 \times 10^{-5}$) to that observed in slices previously incubated with [^{3}H](−)-noradrenaline. Phentolamine increased fractional evoked tritium overflow in a concentration-dependent manner, with a threshold of approximately 1 μM. This is consistent with observations made by other workers in similar tissue preparations (Gothert & Huth 1980; Frankhuyzen & Mulder 1980), the interpretation of such data being that phentolamine blocks a regulation of 5-HT release mediated by endogenous noradrenaline acting at α-adrenoceptors located on 5-HT nerve terminals. However, this interpretation has been challenged on the basis that other α_2-adrenoceptor blockers, such as RX781094 (idazoxan) and yohimbine do not produce the same effect as phentolamine. In the present experiments, concentrations of yohimbine up to 1 μM did in fact increase fractional evoked tritium overflow. This effect could be attributed to blockade by yohimbine of 5-HT autoreceptors, since yohimbine is known to block some 5-HT receptors (Matthews & Smith 1980), but more likely, to blockade of an α-adrenoceptor component regulating cortical 5-HT release under the present experimental conditions. In the present experiments no attempt has been made to characterize pharmacologically these α-adrenoceptors and, given the marginal selectivity of this compound between subtypes of α-adrenoceptors (McGrath 1982), the observed effect could arise from blockade of α_1- or α_2-adrenoceptors (see Ennis 1983). This effect would depend on the concentration of endogenous noradrenaline at the α-adrenoceptors on the 5-HT nerve terminals during electrical stimulation, and hence on the stimulation parameters used, size of brain slice and rate of superfusion. At higher concentrations of yohimbine, a reduction in fractional evoked tritium overflow was observed, compatible with a local anaesthetic action, as had been suggested by Gothert et al (1981). As with the noradrenergic neurons in these slices, this putative local anaesthetic action is slow in onset, indicated by the progressive decline in tritium overflow during the more prolonged exposure used in a single experiment. The ability of yohimbine to inhibit evoked tritium overflow appeared more pronounced in slices pre-incubated with [^{3}H]5-HT than with [^{3}H](−)-noradrenaline. This may also be explained in terms of a local anaesthetic action. Thus, if the stimulus applied to the release mechanism in 5-HT neurons is less suprathreshold than that in noradrenergic neurons, then the increase in stimulus threshold occurring during exposure to yohimbine could have a more pronounced effect on the response to electrical stimulation of 5-HT neurons. Alternatively, yohimbine may be selectively accumulated by, or have additional actions on, the latter neurons.

The possible local anaesthetic action of yohimbine should be taken into account in the interpretation of the effects of the drug on transmitter release (see, for example Kalsner & Chan 1979; Arbilla et al 1982). Moreover, the present results indicate that in superfused, electrically stimulated brain slices, although fractional evoked tritium overflow may remain constant over a number of stimulation periods, the tissue is not necessarily functionally stable during the whole of this time and experiments should be kept to a minimum duration.

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