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THE EFFECTS OF AMITRIPTYLINE, MIANSERIN, PHENOXYBENZAMINE AND PROPRANOLOL ON THE RELEASE OF NORADRENALINE IN THE RAT BRAIN *IN VIVO*

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Abstract—The post synaptic adrenoceptor antagonists, phenoxybenzamine and propranolol, increase the concentration of normetanephrine in the amygdaloid cortex following their acute administration. However, following their chronic administration phenoxybenzamine had no effect, while propranolol decreased the concentrations of this metabolite in the amygdaloid cortex. The chronic effect of propranolol might be attributable to a blockade of pre-synaptic β-receptors. The acute administration of the pre-synaptic α-adrenoceptor agonist clonidine decreases the concentration of normetanephrine in the rat amygdaloid cortex. Conversely, the blockade of pre-synaptic adrenoceptors by yohimbine increases the normetanephrine concentration. The anti-depressants amitriptyline and mianserin also increase normetanephrine concentrations. When given in combination with clonidine, both yohimbine and mianserin antagonise the clonidine induced decrease in normetanephrine concentrations. Evidence suggests that the antagonism of the effect of clonidine is due to the ability of the drugs to block pre-synaptic α-receptors in this brain region. Amitriptyline, which increases normetanephrine concentrations by decreasing the re-uptake of noradrenaline from the synaptic cleft, does not antagonise the effect of clonidine, thereby providing evidence that it does not affect noradrenaline release by acting on pre-synaptic neurotransmitter release mechanisms.

The regulation of central sympathetic activity is thought to involve both pre- and post-synaptic adrenoceptors [1]. It has been proposed [2, 3] that the release of noradrenaline from central neurones is regulated, in part by feedback mechanisms involving pre-synaptic receptors. Thus, the antihypertensive activity of clonidine has been attributed to its ability to stimulate pre-synaptic receptors in the posterior hypothalamus thereby diminishing the calcium mediated exocytosis of noradrenaline [4, 5]. Conversely the α-adrenolytic agent yohimbine, which preferentially blocks pre-synaptic α-receptors, interrupts this feedback loop and hence augments the secretory response to stimulation [6, 3].

According to the catecholamine hypothesis of depression, drugs which increase the inter-synaptic concentration of noradrenaline could be expected to possess anti-depressant properties. *In vitro* evidence suggests that the tetracyclic antidepressant mianserin exerts its antidepressant properties via blockade of central pre-synaptic receptors [7]. The aim of the present study was to examine the affects of pre- and post-synaptic adrenoceptor agonists and antagonists *in vivo* with a view to understanding the mechanism of action of mianserin.

The experimental approach used to evaluate changes in noradrenaline release from the nerve terminal was the determination of changes in concentration of its principal extraneuronal metabolite, normetanephrine. Any alteration in the concentration of this metabolite could be due to changes in the release of re-uptake of the neurotransmitter. It has previously been shown [8] that pre-treatment with the tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine (αMPT) blocks any effects on noradrenaline synthesis and release due to feedback regulation from the post-synaptic receptor site. Any changes in concentration of normetanephrine in the presence of αMPT are therefore likely to reflect activity at pre-synaptic rather than post-synaptic receptor sites.

Part of this study was first communicated to the Summer Meeting of the British Association of Psychopharmacology, Cardiff, July 1978[8].

MATERIALS AND METHODS

Male Wistar rats (200–220 g) were used in all experiments. The animals were housed under normal animal house conditions until the commencement of the experiment and then randomly assigned to cages in groups of five. In each experiment normetanephrine concentrations were determined fluorimetrically using the methods of Anton and Sayre [9]. The investigation was restricted to studying changes within the amygdaloid cortex, an area of brain known to contain noradrenergic nerve terminals and also to be an integral part of the limbic system whose function may be impaired in depression. The first experiment was aimed at ascertaining optimum doses of the five drugs, propranolol, phenoxybenzamine, amitriptyline, clonidine and yohimbine for use in subsequent experiments. Rats were injected intraperitoneally with physiological saline (control group), amitriptyline (2.5, 5.0 or 10 mg/kg for one hr), clonidine (1, 2.5 or 5 mg/kg for two hr), yohimbine (1, 2.5 or 5 mg/kg for one hr), propranolol (2, 5, 10, 12, 15 mg/kg for one hr) or phenoxybenzamine (2, 5, 10, 12, 15 mg/kg for one hr). Due to its water insolubility,

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phenoxybenzamine was dissolved in 70% (v/v) ethyl alcohol containing 10% N HCl neutralized with NaOH to pH 7.5 and then diluted with distilled water. The solution was freshly prepared prior to each experiment.

The period of pretreatment was chosen to co-incide with the peak effect of the compounds on brain monoamine turnover [10, 12]. Due to the slight hypothermia occurring following treatment with yohimbine, rats injected with this drug were maintained in an ambient temperature of 37° throughout the period of treatment.

The chronic effect of propranol and phenoxybenzamine were also assessed. Groups of 5 rats were injected once daily (16:00 hr) with 10 mg/kg intraperitoneal phenoxybenzamine or 12 mg/kg i.p. propranolol for 14 days. Controls were injected with physiological saline. All animals were decapitated 18 hr after the last injection and the normetanephrine content of the amydaloid cortex and mid-brain determined.

In the second experiment, groups of five rats were injected intraperitoneally with physiological saline (control group), αMPT methyl ester (400 mg/kg for 3 hr), amitriptyline (10 mg/kg for 1 hr), clonidine (2.5 mg/kg for 2 hr), mainserin (15 mg/kg for 1 hr), yohimbine (1 mg/kg for 2 hr), propranolol (12 mg/kg for 1 hr), phenoxybenzamine (10 mg/kg for 1 hr), or with a combination of αMPT and one of the other drugs. All rats were killed three hours after administration of the tyrosine hydroxylase inhibitor.

In the third experiment, groups of five rats were injected intraperitoneally with physiological saline (control group), clonidine, amitriptyline, mianserin or a combination of clonidine and either amitriptyline or mianserin. Clonidine was injected 30 min before mianserin or amitriptyline and the rats killed one hour later.

In all cases rats were killed by stunning and decapitation, followed by immediate (within 15 sec) exposure of head to 1.5 sec of microirradiation in a 2.8 kW oven [13]. Use of microwave irradiation for the rapid inactivation of brain enzymes prior to assay is an accepted technique in neurotransmitter determinations [14, 15]. The amygdaloid cortex was removed and dissected according to the method described by Tonge [16]. Results are expressed as mean ± standard error and were analysed according to the Student's *t* test.

RESULTS

The pre-synaptic α-receptor blocking drug yohimbine and the tricyclic antidepressant amitriptyline significantly increased the concentration of normetanephrine in the amygdaloid cortex of the rat as did the α- and β-adrenolytic agents (phenoxybenzamine) and (propranolol) (Table 1). The maximal rise in normetanephrine concentrations was obtained with 1 mg/kg yohimbine, 10 mg/kg amitriptyline and phenoxybenzamine and 12 mg/kg propranolol. Doses of yohimbine greater than 1 mg/kg caused a decrease in the normetanephrine concentration. Conversely, the pre-synaptic receptor agonist clonidine decreased the concentration of normetanephrine (Table 1); 2.5 mg/kg clonidine produced an almost maximal effect without causing appreciable hypothermia. From this preliminary experiment doses of clonidine, yohimbine, amitriptyline, propranolol and phenoxybenzamine were chosen for subsequent studies.

Table 1. Normetanephrine concentrations in amygdaloid cortex following acute administration of clonidine, yohimbine, amitriptyline, propranolol and phenoxybenzamine

Drug	Dose in mg/kg	Concentration of normetanephrine (nmoles/g)
Control	0	0.64 ± 0.02
Amitriptyline	2.5	0.69 ± 0.02
Treatment time: 1 hr	5	0.75 ± 0.06*
	10	0.82 ± 0.05*
Control	0	0.68 ± 0.05
Clonidine	1	0.60 ± 0.07
Treatment time: 2 hr	2.5	0.48 ± 0.05*
	5	0.45 ± 0.03*
Control	0	0.68 ± 0.03
Yohimbine	1	0.79 ± 0.04*
Treatment time: 1 hr	2.5	0.69 ± 0.07
	5	0.52 ± 0.06
Control	0	0.71 ± 0.04
Propranolol	2	0.72 ± 0.06
Treatment time: 1 hr	5	0.75 ± 0.03
	10	0.81 ± 0.06
	12	0.83 ± 0.02*
	15	0.70 ± 0.05
Phenoxybenzamine	0	0.71 ± 0.04
Treatment time: 1 hr	2	0.68 ± 0.05
	5	0.83 ± 0.02*
	10	0.90 ± 0.03*
	12	0.92 ± 0.06*
	15	0.87 ± 0.04*

* Difference between control and drug treated group significant at $P < 0.05$.

Following the chronic administration of propranolol or phenoxybenzamine, no change in the concentration of normetanephrine was found in the mid-brain region (Table 2). However, prolonged propranolol treatment resulted in a significant decrease (27 per cent) in the concentration of this metabolite; phenoxybenzamine had no effect on this brain region.

In the second experiment (Table 3), the tyrosine hydroxylase inhibitor αMPT reduced the concentration of normetanephrine. In this experiment αMPT pretreatment was used in combination with other drugs in order to block any effects they might have on noradrenaline synthesis and release via their actions on the post-synaptic receptors. It was found that the rise in normetanephrine concentrations produced by yohimbine; amitriptyline and mianserin were unaffected by prior administration of αMPT. Pretreatment with the tyrosine hydroxylase inhibitor did, however, prevent the increase in normetanephrine concentrations produced by propranolol and phenoxybenzamine.

Table 2. Chronic effects of propranolol and phenoxybenzamine on the normetanephrine content of the amygdaloid cortex and mid-brain

Group	Amygdaloid cortex	Mid-brain
Controls	0.71 ± 0.07	0.43 ± 0.03
Phenoxybenzamine	0.72 ± 0.04	0.40 ± 0.05
Propranolol	0.52 ± 0.04*	0.40 ± 0.02

* Difference from controls significant at $P < 0.05$.

Table 3. Normetanephrine concentrations in the amygdaloid cortex following acute treatment with pre-synaptic receptor stimulants and blockers

	Concentration of normetanephrine (nmoles/g)
Control	0.62 ± 0.02
α-Methyl-*p*-tyrosine (400 mg/kg i.p.)	0.40 ± 0.03*
Clonidine	0.49 ± 0.03*
Clonidine + αMPT	0.50 ± 0.04
Yohimbine	0.78 ± 0.03*
Yohimbine + αMPT	0.71 ± 0.03†
Amitriptyline	0.79 ± 0.09*
Amitriptyline +αMPT	0.72 ± 0.04†
Mianserin	1.04 ± 0.08*
Mianserin + αMPT	1.13 ± 0.02†
Phenoxybenzamine	0.83 ± 0.03*
Phenoxybenzamine + αMPT	0.35 ± 0.01
Propranolol	0.79 ± 0.02*
Propranolol + αMPT	0.45 ± 0.06

* Difference between control and drug-treated significant at $P < 0.05$.

† Difference between αMPT and αMPT + drug significant at $P < 0.05$.

In the third experiment, the effects of yohimbine and the two antidepressants mianserin and amitriptyline on the reduction in normetanephrine concentrations caused by clonidine were investigated. Yohimbine and mianserin were found to reverse the decrease in the concentration of normetanephrine (Table 4). The effect of amitriptyline was unaffected by clonidine pre-treatment.

DISCUSSION

The results of the investigation show that stimulation of the pre-synaptic α-adrenoceptors by the α-agonist clonidine [12], reduces the concentration of the extra-neuronally formed noradrenaline metabolite, normetanephrine, in the amygdaloid cortex. Conversely, blockade of the pre-synaptic receptor by yohimbine [17] increases the release of noradrenaline from the nerve terminal and this is reflected by the increase in concentration of normetanephrine. However, if it is assumed that normetanephrine is formed primarily in the synaptic cleft, an increase in its concentration could also reflect an increase in synaptic concentrations of transmitter in response to reduced re-uptake or increased release via activation of post-synaptic feedback mechanisms.

Table 4. Changes in the normetanephrine concentration of the amygdaloid cortex in response to yohimbine, mianserin and amitriptyline and their interactions with clonidine

	Normetanephrine concentration (nmoles/g)
Controls	0.71 ± 0.06
Clonidine	0.44 ± 0.04*
Clonidine + mianserin	0.89 ± 0.04*†
Mianserin	1.09 ± 0.04*
Clonidine + yohimbine	0.75 ± 0.05†
Yohimbine	0.86 ± 0.03*
Clonidine + amitriptyline	1.11 ± 0.10*†
Amitriptyline	0.92 ± 0.08*

* Difference between control and drug-treated groups significant at $P < 0.05$.

† Difference between clonidine and clonidine + drug-treated group significant at $P < 0.05$.

Following the acute administration of the adrenergic receptor blockers phenoxybenzamine and propranolol, reported to act preferentially on post-synaptic receptors, the normetanephrine content of the amygdaloid cortex is increased. This appears to confirm the view of other investigators [17, 18] that blockade of either post-synaptic α- or β-adrenoceptors enhances noradrenaline synthesis and release, presumably via feedback stimulation of tyrosine hydroxylase activity [19]. That this increase in normetanephrine concentrations is prevented by prior administration of αMPT clearly indicates that the inhibition of tyrosine hydroxylase activity eliminates possible feedback effects on the synthesis and release of noradrenaline via post-synaptic receptor blockade.

The effects of the chronic administration of propranolol and phenoxybenzamine on the concentration of normetanephrine differ from those changes occurring after acute administration. Thus phenoxybenzamine causes no significant change in the normetanephrine concentration after chronic administration whereas propranolol causes a slight, but statistically significant, decrease in the normetanephrine content of the amygdaloid cortex. Neither drug caused any change in the normetanephrine content of the mid-brain. These results suggest that following prolonged administration of phenoxybenzamine, a decreased sensitivity of the post-synaptic α-adrenoceptors occurs, thereby reducing the feed-back stimulation of tyrosine hydroxylase activity from the post-synaptic receptor site. However, prolonged blockade of β-adrenoceptors by propranolol results in a decreased noradrenaline release. One possible explanation for this effect could be that following chronic administration, propranolol inhibits pre-synaptic β-adrenoceptors as well as the post synaptic receptors.

Pretreatment with αMPT had no effect on the response of normetanephrine concentrations to the administration of either clonidine or yohimbine. This finding supports those of others [1, 12] that stimulation of central pre-synaptic α-adrenoceptors by clonidine reduces noradrenaline release whereas blockade of these receptors stimulates release.

The observation that yohimbine antagonises the reduction in normetanephrine concentration caused by clonidine is consistent with those of other investigators who found that acutely administered yohimbine antagonises both the analgesic and sedative action of clonidine [21] and its suppression of paradoxical sleep [22].

The antidepressant drugs mianserin and amitriptyline alone and in the presence of αMPT, both increase the normetanephrine concentrations in the amygdaloid cortex. It was anticipated that amitriptyline would increase normetanephrine concentration by prolonging

intra-neuronal catabolism as a consequence of its effects on re-uptake. Mianserin, on the other hand, has no effect on uptake *in vivo* [23] but has been shown to reduce noradrenaline release from pre-loaded synaptosomes possibly by antagonizing pre-synaptic α-adrenoceptors [24]. The increase in normetanephrine concentrations found after mianserin plus αMPT in the present study are therefore interpreted as reflecting an increased release of noradrenaline due to blockade of pre-synaptic α-receptors i.e. a yohimbine-like effect.

The finding that αMPT has no effect on the mianserin-induced increase in normetanephrine concentrations discounts any possible post-synaptic site of action. The hypothesis that mianserin increases noradrenaline release *in vivo* is consistent with the increase in noradrenaline turnover found by Kafoe and Leonard [23, 25] after mianserin administration. Conventional tricyclic antidepressants such as amitriptyline reduce amine turnover [26], presumably as a consequence of their blockade of the re-uptake mechanism with subsequent reduction in synthesis via feedback regulation [27].

Further evidence that mianserin acts on pre-synaptic receptors comes from its ability to antagonise the effect of clonidine on normetanephrine concentrations in a similar manner to yohimbine. This suggests that both drugs are acting on the same pre-synaptic receptors. The effects of amitriptyline is unaltered by clonidine pre-treatment. It follows that if the increase in normetanephrine concentrations produced by amitriptyline is unrelated to pre-synaptic receptor blockade, pretreatment with clonidine should have little, if any, antagonistic effect. Indeed, amitriptyline produces a similar increase in normetanephrine concentrations in the presence and absence of clonidine. The effects of amitriptyline and mianserin are thus qualitatively distinguishable.

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