Br. J. Pharmac. (1978), **64**, 259–264.

DIFFERENCES BETWEEN PRESYNAPTIC AND POSTSYNAPTIC α-ADRENOCEPTORS IN THE ISOLATED NICTITATING MEMBRANE OF THE CAT: EFFECTS OF METANEPHRINE AND TOLAZOLINE

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1 The α-adrenoceptor blocking agent, tolazoline, and the *O*-methylated metabolite of adrenaline, metanephrine, produced a concentration-dependent increase of tension in the smooth muscle of the cat isolated nictitating membrane. These effects were considered to be due to the activation of postsynaptic α-adrenoceptors.

2 The responses to nerve stimulation of this muscle were neither potentiated nor blocked by tolazoline (0.1 to 10 μM) or metanephrine (1 to 10 μM).

3 ^{3}H-transmitter overflow evoked by electrical stimulation was not modified by tolazoline or metanephrine in concentrations in which these drugs stimulated the postsynaptic α-adrenoceptors.

4 Since tolazoline and metanephrine failed to activate the presynaptic α-adrenoceptors of the cat nictitating membrane under experimental conditions in which they stimulated the postsynaptic α-adrenoceptors, these results further support the view that the presynaptic (α_2) adrenoceptors differ from the postsynaptic (α_1) adrenoceptors.

Introduction

The release of noradrenaline elicited by nerve stimulation is regulated through a negative feed-back mechanism which is mediated by presynaptic α-adrenoceptors (for reviews see Langer, 1974; 1977; Starke, 1977). Activation of presynaptic α-adrenoceptors leads to a decrease in transmitter release during nerve stimulation, while blockade of these receptors enhances the release of noradrenaline. Differences between the presynaptic α-adrenoceptor that regulates noradrenaline release and the postsynaptic α-adrenoceptor which mediates the response of the effector organ were first postulated by Langer (1973). Subsequently additional reports confirmed that the affinity of α-receptor agonists and antagonists was different when the presynaptic and the postsynaptic α-adrenoceptors were compared in several tissues of different species (Dubocovich & Langer, 1974; Cubeddu, Barnes, Langer & Weiner, 1974; Starke, Montel, Gayk & Merker, 1974; Starke, Borowsky & Endo, 1975; Drew, 1976). However, in the cat nictitating membrane it was reported that for a wide range of concentrations of phenoxybenzamine there was a good correlation between the block of the postsynaptic responses and the enhancement in noradrenaline release during nerve stimulation (Enero, Langer, Rothlin & Stefano, 1972). These results were compatible with the view that in this tissue the differences between pre- and postsynaptic α-adrenoceptors may not exist or may be very small.

The aim of the present experiments was to compare the effects on the pre- and postsynaptic receptors of the cat nictitating membrane of metanephrine, the *O*-methylated metabolite of adrenaline, which is a potent α-receptor agonist in this tissue (Langer & Rubio, 1973) and of tolazoline, which in the smooth muscle of the cat nictitating membrane stimulates the postsynaptic α-receptor (Hoszowska-Owczarek, Langer, Djordjevic & De Schaepdryver, 1968), although it is more usually an antagonist at this type of receptor.

Methods

Isolated nictitating membrane of the cat

Cats of 2.0 to 4.0 kg body weight and of either sex were anaesthetized with sodium pentobarbitone (35 mg/kg i.p.) and the trachea was cannulated. The eyeball was excised and the nictitating membrane with all the adjoining tissue was placed in slightly modified Krebs solution previously equilibrated with 95% O_2 and 5% CO_2. The composition of the Krebs solution was as follows (mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 2.6, $MgCl_2$ 1.2, NaH_2PO_4 1.0, $NaHCO_3$ 25.0, glucose 11.0, sodium ethylenediamine tetraacetic acid (EDTA) 0.004 and ascorbic acid 0.11.

The medial muscle was dissected free with the postganglionic sympathetic fibres arising from the infratrochlear nerve which innervates this smooth muscle of the nictitating membrane. The cartilage on which the fibres of the medial muscle are inserted was fixed at the bottom of a 5 ml organ bath. The upper end of the muscle was connected to a Grass FTO3C force displacement transducer and the tension developed by the muscle was recorded on a Grass Model 7D Polygraph. The temperature was maintained at 37°C and the organ bath was bubbled with a 95% O_2 and 5% CO_2 mixture. The infratrochlear nerve was pulled through shielded bipolar platinum electrodes for stimulation with monophasic square pulses of 0.5 ms duration and supramaximal voltage delivered by a Grass S88 stimulator. A period of 30 min was allowed to elapse before starting the experiment. During this period the Krebs solution was replaced every 10 min. The resting tension of the muscle was repeatedly adjusted to 2.5 g and it reached steady conditions after 30 to 40 min.

Thirty min after the smooth muscle was set up in the organ bath it was incubated for 30 min with 50 µCi (10 µCi/ml: 0.64 µM) of (±)-[7-^{3}H]-noradrenaline (The Radiochemical Centre, Amersham, sp. act. 15.7 Ci/mmol). Then, the tissue was washed eight times, for 1 min each time with fresh Krebs solution. Subsequently, the Krebs solution was replaced at 10 min intervals for the next 30 min and thereafter at 5 min intervals for a further 30 min period.

The spontaneous outflow of radioactivity from the tissue into the bathing fluid was monitored by counting 1 ml samples of the fluid which had been in contact with the tissue for 5 min. Collection of samples for total radioactivity began 60 min after the end of the incubation with [^{3}H]-noradrenaline. Whenever drugs were added to the organ bath, they were replaced with each renewal of the bathing fluid. The nerves were stimulated at 4 Hz for 5 min with the previously mentioned parameters. Two periods of nerve stimulation were applied with an interval of 30 min.

The outflow of radioactive products was measured before, during and after the period of nerve stimulation. The overflow of total radioactivity induced by nerve stimulation was calculated by subtraction of the spontaneous outflow assumed to have occurred in each sample during and after the period of nerve stimulation, its value being the basal resting release obtained in the 5 min period immediately before stimulation. The 'total overflow of the transmitter' was the sum of all increases above the resting levels induced by the period of nerve stimulation. The overflow of radioactivity elicited by each period of stimulation was expressed as a fraction of the total radioactivity remaining in the tissue at the start of stimulation (Langer & Enero, 1974).

Total radioactivity remaining in the smooth muscle of the nictitating membrane was determined at the end of each experiment. The tissues were blotted dry, weighed and solubilized in 1 ml Soluene (Packard) which was brought to 5 ml by the addition of 0.4 N perchloric acid. Samples of 1 ml of the solubilized tissue or of the bathing solution which had been in contact with the nictitating membrane for 5 min were collected and added to 7 ml of the scintillator mixture, Scintix, from Isotec. Concentration-effect curves for tolazoline, metanephrine or (−)-noradrenaline were determined by cumulative addition of the drug so that the final concentration in the bath was increased by a factor of about 3 whenever a steady response to the previous concentration was achieved. Only one concentration-effect curve was determined in each preparation.

Statistical calculations were performed according to conventional procedures (Snedecor & Cochran, 1967).

The following drugs were used: tolazoline hydrochloride (Ciba), (±)-metanephrine hydrochloride (Sigma) and (−)-noradrenaline bitartrate monohydrate (Sigma). Concentrations of the drugs are expressed on a molar basis.

Results

Postsynaptic effects of tolazoline and metanephrine on the cat isolated nictitating membrane

Tolazoline produced a concentration-dependent increase in tension of the smooth muscle of the nictitating membrane although even at the highest concentration (30 µM) the response was no more than one third of the maximum produced by noradrenaline (Figure 1a and b). These responses are due to the activation of α-adrenoceptors, because they are blocked by phentolamine under *in vivo* conditions (Hoszowska-Owczarek *et al.*, 1968) and also under our *in vitro* conditions. Metanephrine, the *O*-methylated metabolite of adrenaline also behaved as an agonist on the postsynaptic α-receptors of the cat nictitating membrane and produced a maximum response similar in amplitude to that elicited by noradrenaline (Figure 1a and b). As with tolazoline, the responses of the smooth muscle of the nictitating membrane to metanephrine are due to the activation

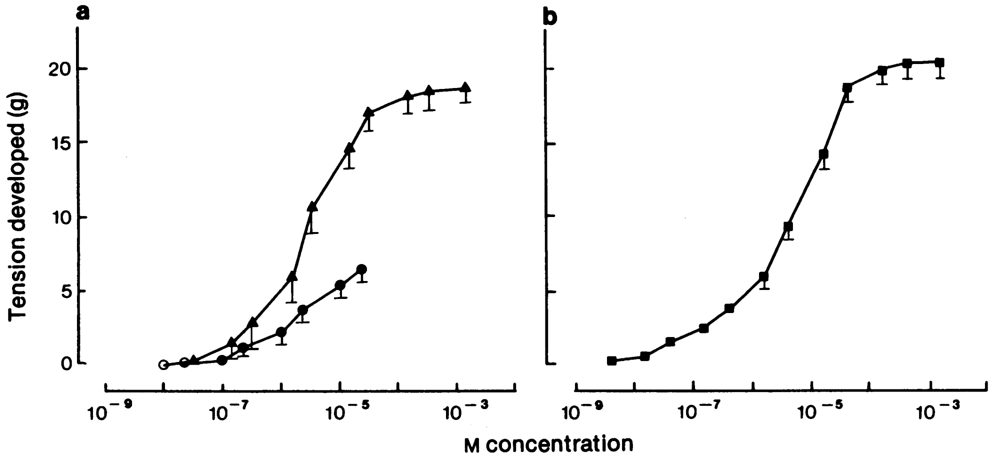

Figure 1 Stimulation of the postsynaptic α-adrenoceptors in the cat isolated nictitating membrane by noradrenaline, metanephrine and tolazoline. Ordinates: development of tension in g; abscissae: molar concentration of drugs. (a) Effects of (±)-metanephrine (▲, $n = 8$) and tolazoline (●, $n = 9$). (b) Effects of (−)-noradrenaline (■, $n = 20$). Mean values are shown; vertical lines indicate s.e. means.

of postsynaptic α-receptors, because they are blocked by phentolamine (Langer & Rubio, 1973).

Effects of tolazoline and metanephrine on the responses to nerve stimulation of the cat isolated nictitating membrane

In the absence of drugs, there was no difference between the postsynaptic responses of the nictitating membrane to two consecutive periods of nerve stimulation. In the presence of concentrations of tolazoline which did not themselves elicit a contraction of the nictitating membrane (0.1 and 1 μM), the responses to nerve stimulation remained unaffected (see ratio S_2 over S_1, Table 1). When 10 and 30 μM tolazoline were employed there was a contraction of the nictitating membrane before S_2 (Table 1). Because it was previously shown that in the partially contracted nic-

Table 1 Effects of tolazoline and metanephrine on responses to nerve stimulation of the isolated nictitating membrane of the cat

Experimental group	*n*	*S_1 (g)*	*Tone (g)*	*S_2 (g)*	*S_2/S_1*
Control	13	7.42 ± 0.69	—	7.75 ± 0.74	1.05 ± 0.03
Tolazoline					
0.1 μM	7	8.00 ± 1.05	—	8.64 ± 1.34	1.06 ± 0.06
1.0 μM	5	7.30 ± 1.11	0.50 ± 0.12	7.40 ± 1.45	0.98 ± 0.06
10.0 μM	11	6.75 ± 0.64	4.05 ± 1.72	8.11 ± 0.06	1.25 ± 0.08*
30 μM	11	6.38 ± 0.85	6.82 ± 0.64	8.65 ± 0.83	1.46 ± 0.12**
Metanephrine					
1 μM	15	7.11 ± 0.64	4.43 ± 0.39	8.85 ± 0.70	1.33 ± 0.11*
3 μM	5	6.13 ± 0.16	6.18 ± 0.82	8.72 ± 1.43	1.53 ± 0.12**
10 μM	18	5.86 ± 0.38	8.25 ± 0.46	10.07 ± 0.51*	1.87 ± 0.18***

Values are mean ± s.e. mean; n = number of experiments. S_1: maximal development of tension (g) (first period of nerve stimulation); Tone: tension developed during exposure to tolazoline or metanephrine; S_2: maximal development of tension (g) (second period of nerve stimulation). The interval between the two periods of nerve stimulation (4 Hz, for 5 min, square pulses of 0.5 ms duration and supramaximal voltage) was 30 min. Tolazoline was added to the organ bath 25 min before S_2, and metanephrine 10 min before S_2.

*$P < 0.05$; **$P < 0.005$; ***$P < 0.001$ when compared with control.

titating membrane the actual response to an agonist is more accurately estimated by the addition of the observed response to the underlying tone (Langer, 1966), the responses to nerve stimulation were calculated in the same way. Table 1 shows that the total responses to nerve stimulation obtained in the presence of 10 and 30 μM tolazoline were significantly increased.

With the concentrations of metanephrine employed (1, 3 and 10 μM) there was always an underlying tone before the second period of nerve stimulation and in each group the *total* response was significantly increased in a concentration-dependent manner (Table 1).

The increase in responses to nerve stimulation observed in the presence of tolazoline and metanephrine do not reflect a true potentiation but only additive effects of the underlying tone and the actual response (Langer, 1966). Similarly, additive effects of metanephrine on responses to nerve stimulation and to exogenous noradrenaline were reported for the cat nictitating membrane *in vivo* (Langer, Bogaert & De Schaepdryver, 1967).

Effect of tolazoline and metanephrine on ³H-transmitter overflow elicited by nerve stimulation of the isolated nictitating membrane.

In the controls there was no difference between the overflow of ³H-neurotransmitter elicited by two consecutive periods of nerve stimulation (Table 2). Exposure to tolazoline (0.1 to 30 μM) did not significantly modify the overflow of [³H]-noradrenaline elicited by nerve stimulation although with concentrations of 10 and 30 μM there was a small, but not statistically significant, increase in overflow (Table 2). Not only did this small increase in overflow not reach levels of statistical significance but it also failed to show concentration-dependent characteristics (Table 2). The spontaneous outflow of radioactivity before S_2 was not affected by any of the concentrations of tolazoline employed.

As shown in Table 2, there was no significant effect on ³H-transmitter overflow when nerve stimulation was applied in the presence of 1, 3 or 10 μM metanephrine. The spontaneous outflow of radioactivity was not affected during exposure to these concentrations of metanephrine.

Discussion

The present experiments have confirmed earlier observations that the α-receptor blocking agent, tolazoline, and the *O*-methylated metabolite of adrenaline, metanephrine, share the unusual property of stimulating postsynaptic α-adrenoceptors in the smooth muscle of the cat nictitating membrane (György, 1957; Hoszowska-Owczarek *et al.*, 1968; Langer & Rubio, 1973).

Both drugs show unexpected effects in the nictitating membrane as tolazoline is a competitive α-receptor blocking agent in most tissues (Goodman & Gilman, 1975) and metanephrine is a metabolite of adrenaline with very low potency for the postsynaptic α-adrenoceptors in other tissues (Champagne, D'Iorio

Table 2 Effects of tolazoline and metanephrine on ³H-transmitter overflow elicited by nerve stimulation of the isolated nictitating membrane of the cat

Experimental group	*n*	*Fraction released per shock (× 10⁻⁵) (a)* S_1	S_2	*Ratio* S_2/S_1
Control	13	1.51 ± 0.24	1.60 ± 0.26	1.06 ± 0.10
Tolazoline				
0.1 μM	6	1.18 ± 0.07	1.18 ± 0.25	0.99 ± 0.17
1 μM	5	1.64 ± 0.48	1.29 ± 0.26	0.88 ± 0.09
10 μM	10	1.40 ± 0.20	2.01 ± 0.40	1.58 ± 0.29
30 μM	6	1.68 ± 0.27	2.30 ± 0.37	1.46 ± 0.19
Metanephrine				
1 μM	8	1.53 ± 0.36	1.22 ± 0.25	1.01 ± 0.29
3 μM	5	1.39 ± 0.34	1.82 ± 0.60	1.25 ± 0.22
10 μM	10	1.88 ± 0.44	1.19 ± 0.19	0.78 ± 0.11

Values are mean ± s.e. mean; *n* = number of experiments. (a): Fraction of the total radioactivity released per shock during the period of nerve stimulation (4 Hz, for 5 min, square pulses of 0.5 ms duration and supramaximal voltage). S_1 corresponds to the first period of nerve stimulation and S_2 to the second one, applied 30 min after S_1. Tolazoline was added to the organ bath 25 min before S_2 and metanephrine 10 min before S_2.

& Beaulnes, 1960; Pruss, Maengwyn-Davies & Wurzel, 1965). If the presynaptic α-adrenoceptors of the nictitating membrane were identical with the postsynaptic α-adrenoceptors then both tolazoline and metanephrine would be expected to decrease ^{3}H-transmitter release elicited by nerve stimulation in the range of concentrations in which they stimulate the postsynaptic α-receptors. This was clearly not the case under our experimental conditions.

Metanephrine inhibits extraneuronal uptake of noradrenaline (Iversen, 1967; Gillespie, Hamilton & Hosie, 1970; Draskoczy & Trendelenburg, 1970) and it could be argued that this effect may have *per se* enhanced transmitter overflow, thereby masking an actual decrease in [^{3}H]-noradrenaline release resulting from stimulation of presynaptic α-adrenoceptors by this agent. However, in this isolated tissue, inhibition of extraneuronal uptake by hydrocortisone does not affect ^{3}H-transmitter overflow elicited by nerve stimulation (Luchelli-Fortis & Langer, 1975) and furthermore, concentrations of metanephrine of 10 μM or higher are required to inhibit extraneuronal uptake of noradrenaline (Draskoczy & Trendelenburg, 1970). Therefore, inhibition of extraneuronal uptake by metanephrine cannot account for the failure of this agent to reduce ^{3}H-transmitter overflow during nerve stimulation. In addition, neither tolazoline nor metanephrine inhibit neuronal uptake of noradrenaline (Iversen, 1967; Draskoczy & Trendelenburg, 1970).

If tolazoline were to block rather than stimulate the presynaptic receptors then it would be expected to enhance the stimulation-evoked ^{3}H-transmitter overflow. Although tolazoline appeared to stimulate the postsynaptic receptors there was no indication of presynaptic α-adrenoceptor stimulation; indeed, the small but statistically insignificant increase in ^{3}H-transmitter outflow with the high concentrations (see Table 2) suggests that tolazoline could more readily block rather than stimulate these presynaptic receptors. It is of interest to note that in the presence of phentolamine a 3 fold increase was reported in ^{3}H-transmitter overflow from the cat isolated nictitating membrane and also that clonidine, an α-adrenoceptor agonist, concomitantly increased the muscle tension and reduced significantly the overflow during nerve stimulation (Langer & Luchelli-Fortis, 1977). The failure of both tolazoline and metanephrine to activate the presynaptic receptors under conditions when they appeared to activate the postsynaptic ones, provides evidence that differences exist between these two groups of receptors in the cat nictitating membrane although they may not be differentiated unequivocally by all agonists and antagonists.

Based on the differences between the pre- and the postsynaptic α-adrenoceptors it was proposed to refer to them as α_1 for the postsynaptic α-adrenoceptor that mediates the response of the effector organ and as α_2 for the presynaptic α-adrenoceptor which regulates the release of noradrenaline (Langer, 1974). Additional evidence for the differences between pre- and postsynaptic α-adrenoceptors became available during recent years and has given further support to the subclassification of the α-adrenoceptor as α_1 or postsynaptic and α_2 or presynaptic (Cubeddu *et al.*, 1974; Dubocovich & Langer, 1974; Starke *et al.*, 1974; Starke, Endo & Taube, 1975; Drew, 1976; Doxey, Smith & Walker, 1977).

Our results provide additional evidence in support of the view that there are differences between the pre- and the postsynaptic α-adrenoceptors. On the other hand, our finding further complicates the subclassification of α-adrenoceptors because of tissue and species differences. For instance tolazoline, which in other tissues effectively blocks the α_2-adrenoceptors (Drew, 1976; 1977) failed to do so in the cat nictitating membrane. In addition, it was recently reported that prazosin, a selective α_1-adrenoceptor antagonist, blocks the presynaptic α-adrenoceptors in the heart of the dog but fails to do so in the rat heart (Cavero, Lefevre & Roach, 1977; Roach, Lefevre & Cavero, 1978).

The technical assistance of Miss Cristina Iglesias is gratefully acknowledged. We are also grateful to Miss Christine Contant and Miss Colette Féret for typing the manuscript.

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(*Received February 13, 1978.*
Revised April 28, 1978.)