Br. J. Pharmac. (1980), **69**, 35–40

INFLUENCE OF β-ADRENOCEPTOR BLOCKING AGENTS ON THE TURNOVER RATE OF CARDIAC AND SPLENIC NORADRENALINE IN RATS

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1 The effects of (+) and (±)-propranolol, pindolol, alprenolol, practolol, acebutolol and bretylium were studied on the turnover rate of noradrenaline in heart and spleen of rats.

2 Bretylium (8 mg/kg) greatly reduced the turnover rate of noradrenaline in both organs.

3 (+)-Propranolol (4 and 10 mg/kg) also diminished the turnover rate of noradrenaline, but its effects were smaller than those of (±)-propranolol (4 and 10 mg/kg).

4 Pindolol (300 μg/kg) greatly increased the turnover rate of noradrenaline; this effect was especially important in the spleen.

5 Alprenolol (4 and 10 mg/kg) acebutolol (20 and 40 mg/kg) practolol (10 mg/kg) did not produce any significant change.

6 These effects are compatible with the view that β-adrenoceptor blocking agents may affect noradrenaline release in different manners: anaesthetic properties of some of these drugs and blockade of β_2 prejunctional adrenoceptors produce a diminished release of transmitter, whereas the intrinsic sympathomimetic action of pindolol causes the opposite effect.

Introduction

Despite their long use in the treatment of hypertension, the mechanisms whereby propranolol and other β-adrenoceptor blocking drugs affect noradrenergic transmission are still uncertain. Nevertheless, there is a growing body of evidence that the release of noradrenaline (NA) from sympathetic nerve endings may be modulated by activation or blockade of β-adrenoceptors in the nerve terminal through a positive feedback mechanism (see Langer, 1977).

Moreover, (±)-propranolol inhibits the tyramine-evoked release of NA (Benfey & Varma, 1964) and several β-adrenoceptor blocking agents, but not (+)-propranolol, have recently been shown to reduce both tyrosine hydroxylase and dopamine β-hydroxylase activities in rabbit cervical ganglia (Raine & Chubb, 1977).

The present work was designed to investigate further the mechanisms whereby propranolol exerts its antagonistic effect on NA release, and to examine the influence of several β-adrenoceptor blocking drugs with different non-specific pharmacological properties. Actions of (±)-propranolol, alprenolol, pindolol, practolol and acebutolol were compared with the effects of (+)-propranolol and bretylium on the turnover rate of heart and spleen NA in rats.

Methods

Male Wistar rats, weighing 220 ± 20 g, anaesthetized with pentobarbitone (50 mg/kg, i.p.) were given intravenously 25 μCi of [^{3}H]-(±)-NA (27 Ci/mmol, C.E.N; Belgium) and 1 h later half the total dose of the drugs to be tested. (Table 1). They received the remaining half-dose 180 min later. The total doses used were: (+)-, (±)-propranolol and (±)-alprenolol (4 to 10 mg/kg, i.v.), pindolol (300 μg/kg, i.v.), practolol (10 mg/kg, i.v.), acebutolol (20 to 40 mg/kg, i.v.) and bretylium (8 mg/kg, s.c.). Control animals received 0.9% w/v NaCl solution (saline). Rats were killed by dislocation of the neck, 3, 24 and 48 h after the last administration of drug.

Hearts and spleens were quickly removed, chilled on ice and weighed. Organs were homogenized in 10 ml of an ethanol:water solution (74:26, v/v) with an Ultra-Turrax apparatus. After centrifugation, tritiated and endogenous NA were separated from their metabolites by ion-exchange chromatography on Amberlite CG 50 and adsorption on alumina (Thierry, Javoy, Glowinski & Kety, 1968).

[^{3}H]-NA was assayed by liquid scintillation counting of the eluates from alumina columns. Endogenous NA was determinated on the same eluates by the

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spectrophotofluorimetric method of Anton & Sayre (1962); values were corrected for recovery (70%).

After injection of a very small dose of [^{3}H]-NA, the labelled amine concentration declines as a single exponential (Brodie, Costie, Dlabac, Neff & Smookler, 1966). A plot of log [^{3}H]-NA versus time yields a straight line, the slope of which is 0.434 k, k being the rate constant of amine loss.

Taking advantage of the steady state of the endogenous NA levels and of the distribution of the labelled [^{3}H]-NA throughout the stores of endogenous amine, the turnover rate of NA may be calculated from the product of the constant rate of amine loss and the catecholamine tissue content: k x (NA). The turnover time or half-life may be defined as $0.693/k$.

The values for the tissue levels of [^{3}H]-NA were logarithmically transformed for calculation of linearity of regression, standard error of the regression coefficients and significance of differences between regression coefficients (Snedecor & Cochran, 1967).

Results

In control and treated rats, heart and spleen endogenous NA levels did not change with time. Therefore mean values corresponding to steady state levels were calculated for each treatment and for the pooled control series (Figure 1 and Tables 2 & 3).

The changes in the turnover rates paralleled the variations of the rate constants of amine efflux. Comparison of these constants indicates that, in cardiac tissue, pindolol moderately accelerated the turnover rate (+14%) while (±)-propranolol (−34.6 and −43.6%), bretylium (−21%) and (+)-propranolol (±18.3 and −12.6%) lowered it. By contrast, alprenolol, practolol and acebutolol did not induce any significant change.

In the spleen, pindolol greatly (+51.7%) and alprenolol barely (+10 and +8.3%) increased the turnover rate of NA whereas bretylium (−38.3%), (±)-propranolol (−25 and −28.3%), (+)-propranolol (−16.6 and −15%) and acebutolol (−6.7 and −5%) decreased it. Practolol did not produce any significant change.

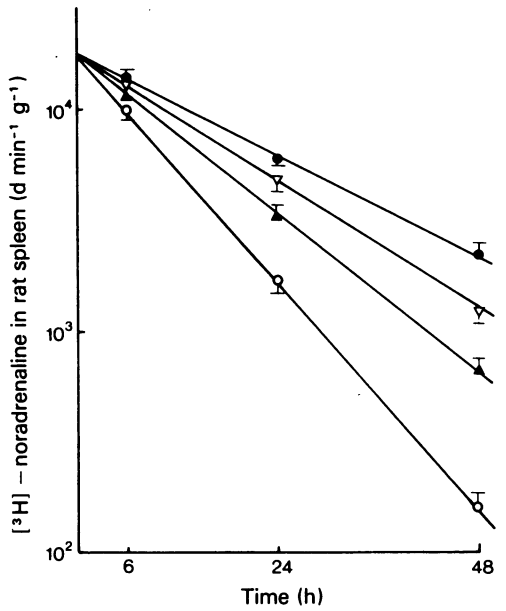

Figure 1 Effect of bretylium (8 mg/kg), (±)-propranolol (10 mg/kg) and pindolol (300 μg/kg) on the turnover rate of spleen noradrenaline. Rats received [^{3}H]-noradrenaline; contents of the tritiated amine were measured in the spleen at various times after this administration either without treatment (controls, ▲, n = 268) or following subsequent treatment by (±)-propranolol (▽, n = 36) bretylium (●, n = 24) or pindolol (○, n = 41). Points represent the mean values; vertical lines show s.e. means.

Table 1 Pharmacological properties of drugs used

Drug	*β-Adrenoceptor antagonism*	*Membrane stabilizing effect*	*Intrinsic stimulating effect*	*Relative β_1 selectivity*
Bretylium	—	+		
(+)-Propranolol	—	+		
(±)-Propranolol	+	+	—	—
(±)-Alprenolol	+	+	+	—
Pindolol	+	—	+	—
Acebutolol	+	+	—	+
Practolol	+	—	+	+

Symbols indicate qualitative properties + present; — absent.

Table 2 Effect of drugs on turnover rate and turnover time of heart noradrenaline (NA)

Treatment	n	*Heart content* ($\mu g/g \pm$ *s.e. mean*)	*Rate constant of amine loss* ($k(h^{-1}) \pm$ *s.e. mean*)	*Turnover rate* ($\mu g\ g^{-1}\ h^{-1}$)	*Change in turnover rate* (%)	*Turnover time* (*h*)
Controls	272	0.87 ± 0.01	0.082 ± 0.001	0.071		8.5
Bretylium 8 mg/kg	25	0.85 ± 0.02	0.067 ± 0.002***	0.056	−21.1	10.3
Pindolol 300 μg/kg	41	0.84 ± 0.01	0.096 ± 0.003**	0.081	+14.0	7.2
(±)-Propranolol						
4 mg/kg	33	0.87 ± 0.02	0.052 ± 0.002***	0.045	−34.6	13.3
10 mg/kg	30	0.85 ± 0.02	0.047 ± 0.002***	0.040	−43.6	14.7
(+)-Propranolol						
4 mg/kg	27	0.85 ± 0.02	0.068 ± 0.003**	0.058	−18.3	10.2
10 mg/kg	20	0.90 ± 0.03	0.069 ± 0.005*	0.062	−12.6	10.0
Alprenolol						
4 mg/kg	25	0.84 ± 0.03	0.084 ± 0.003	0.071	−0.0	8.3
10 mg/kg	25	0.88 ± 0.03	0.083 ± 0.004	0.073	+2.8	8.3
Practolol 10 mg/kg	27	0.87 ± 0.03	0.077 ± 0.003	0.067	−5.6	9.0
Acebutolol						
20 mg/kg	27	0.90 ± 0.04	0.078 ± 0.003	0.070	−1.4	8.9
40 mg/kg	26	0.89 ± 0.04	0.079 ± 0.003	0.070	−1.4	8.8

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 3 Effect of drugs on turnover rate and turnover time of spleen noradrenaline (NA)

Treatment	n	*Spleen content* ($\mu g/g \pm$ s.e. mean)	*Rate constant of amine loss* ($k\ (h^{-1}) \pm$ s.e. mean)	*Turnover rate of NA* ($\mu g\ g^{-1}\ h^{-1}$)	*Change in turnover rate* (%)	*Turnover time* (h)
Controls	268	0.89 ± 0.01	0.068 ± 0.002	0.060		10.2
Bretylium 8 mg/kg	24	0.88 ± 0.02	0.042 ± 0.003***	0.037	−38.3	16.5
Pindolol 300 μg/kg	41	0.93 ± 0.01	0.098 ± 0.003***	0.091	+51.7	7.1
(±)-Propranolol						
4 mg/kg	30	0.86 ± 0.02	0.052 ± 0.002**	0.045	−25.0	13.3
10 mg/kg	36	0.92 ± 0.02	0.047 ± 0.002***	0.043	−28.3	14.7
(+)-Propranolol						
4 mg/kg	23	0.88 ± 0.02	0.057 ± 0.004**	0.050	−16.6	12.2
10 mg/kg	22	0.90 ± 0.02	0.057 ± 0.004**	0.051	−15.0	12.2
Alprenolol						
4 mg/kg	25	0.84 ± 0.02	0.079 ± 0.004*	0.066	+10.0	8.8
10 mg/kg	24	0.85 ± 0.02	0.076 ± 0.004	0.065	+8.3	9.1
Practolol 10 mg/kg	25	0.88 ± 0.02	0.065 ± 0.004	0.057	−5.0	10.7
Acebutolol						
20 mg/kg	25	0.94 ± 0.03	0.060 ± 0.004*	0.056	−6.7	11.6
40 mg/kg	24	0.92 ± 0.03	0.062 ± 0.003*	0.057	−5.0	11.2

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Discussion

The reduction in preganglionic nerve activity (Schmitt, Fénard & Schmitt, 1971; Lewis & Haeusler, 1975), the impairment *in vitro* and *in vivo* of the responses to sympathetic nerve stimulation concomitant with an unchanged or enhanced action of added NA (Day, Owen & Warren, 1968; Barrett & Nunn, 1970; Eliash & Weinstock, 1971; Mylecharane & Raper, 1973), and the decrease in the turnover of heart NA (Lemmer & Saller, 1974) are evidence suggesting, for acutely administered (±)-propranolol, a central and/or prejunctional inhibitory action.

However the mechanism whereby (±)-propranolol exerts its peripheral inhibition is still under discussion. In addition to the presynaptic negative feedback mechanism for NA release by nerve stimulation via α-adrenoceptors, a positive feed-back mechanism in adrenergic nerve endings triggered through the activation of presynaptic β-adrenoceptors has been assumed (Adler-Graschinsky & Langer, 1975; Stärjne & Brundin, 1975). Some results are compatible with this view: cyclic adenosine 3',5'-monophosphate (cyclic AMP) (Wooten, Thoa, Kopin & Axelrod, 1973), isoprenaline and adrenaline (Adler-Graschinsky & Langer, 1975) facilitate the release of NA from adrenergic nerve terminals, while (±)-propranolol lowers it, and, unlike the (+)-isomer, decreases the response to stimulation of lumbar sympathetic nerves (Åblad, Ek, Johansson & Waldeck, 1970). For other investigators (Elias & Weinstock, 1971; Mylecharane & Raper, 1973), the slowly developing reduction in the responses to stimulation of isolated organs associated with largely unchanged responses to NA and the blockade, reversed by (+)-amphetamine and unaffected by washing, strongly suggests a typical guanethidine-like adrenergic blocking effect, at low doses. However, results of other nerve stimulation experiments performed *in vivo* (Dawes & Faulkner, 1975) as well as *in vitro* (Day *et al.*, 1968; Barrett & Nunn, 1970; Hughes & Kneen, 1976) do not fit with this assumption. The reason for this discrepancy is not clear. In large doses, there is a general agreement that the rapid blockade of sympathetic stimulation, reversed by washing and unaffected by (+)-amphetamine, is due to a non specific depressant action linked to its local anaesthetic effect (Day *et al.*, 1968; Barrett & Nunn, 1970; Hughes & Kneen, 1976).

(±)-Propranolol (Barrett & Cullum, 1968) alprenolol (Åblad, Brögard & Ek, 1967) are equipotent β-adrenoceptor antagonists with powerful local anaesthetic activity which cause non specific depression of myocardial function. In contrast to β-blocking activity the (+), and (±)-isomers are equally effective as local anaesthetics (Morales-Aguilera & Vaughan-Williams, 1965; Belliveau & Covino, 1969); (±)-alprenolol, but not (±)-propranolol, possesses intrinsic sympathomimetic activity. On the other hand, pindolol is a very potent antagonist with very weak anaesthetic effect but which exhibits marked sympathomimetic properties (Guidicelli, Schmitt & Boissier, 1969). Of the two cardioselective β-blockers, practolol and acebutolol, the first is less potent than (±)-propranolol and has sympathomimetic properties but no local anaesthetic activity (Dunlop & Shanks, 1968); the second (Basil, Jordan, Loveless & Maxwell, 1973) has a potency comparable with that of practolol, possesses less marked intrinsic sympathomimetic activity than practolol but, like propranolol, has significant local anaesthetic action. Relatively high doses of these drugs were used in order to approach those employed in the treatment of human hypertension.

Drugs with pronounced local anaesthetic activity such as bretylium, (+) and (±)-propranolol, diminished the turnover rate of NA in heart and spleen, suggesting that non-specific properties of (±)-propranolol may in part account for the impairment of NA release. Nevertheless, the inhibitory action of (±)-propranolol is more marked than that of its dextro isomer, suggesting that blockade of central and/or prejunctional β-adrenoceptors may also reduce the efflux of NA from sympathetic nerves. On the contrary, pindolol enhanced the turnover rate of NA, both in heart and spleen. This latter result confirms previous data from Wagner, Reinhardt & Schümann (1973) and Leonard (1972) who also showed the capacity of pindolol to enhance NA release from heart and brain, respectively. Our results confirm that drugs with anaesthetic properties depress the liberation of NA at a prejunctional site. On the other hand, they provide evidence for the view that activation of presynaptic β-adrenoceptors, due to the intrinsic sympathomimetic action of β-blockers such as pindolol, results in an acceleration of NA release.

This fits well with the hypothesis of a positive feedback mechanism in adrenergic nerve endings (Langer, 1977). It may therefore be suggested that the overall effect on the release of NA of all β-blockers, at any dose, depends on opposite influences: two depressant actions linked to anaesthetic activity and to blockade of prejunctional β-adrenoceptors, and a facilitory one related to intrinsic sympathomimetic activity. Failure of alprenolol to produce marked effects on the turnover rate of NA is interpretated as the result of the sum of contrary effects, due to opposite properties of this drug.

The β_1 or β_2 nature of the prejunctional β-adrenoceptors is still somewhat controversial. In the rat portal vein, these receptors were blocked by metoprolol (Dahlöf, Åblad, Borg, Ek & Waldeck, 1975), which suggests that they are of the β_1-type. Nevertheless, according to Stärjne & Brundin (1976) the facilitation of NA release involves β_2-adrenoceptors since the β_1 agonist, H 110/38, had no effect, whereas salbutomol

and terbutaline enhanced transmitter release. Our results, showing that two β_1-adrenoceptor blocking agents, acebutolol and practolol had no effect on the turnover rate of heart and spleen NA, fit well with the latter suggestion.

Our work suggests that β-blockers may depress NA release, partially through non-specific properties, but also by blockade of β_2 prejunctional adrenoceptors. Since pindolol, which had an opposite effect, and acebutolol and practolol, which were ineffective, have been reported, like all β-adrenoceptor blocking agents, to exhibit antihypertensive activity, in contrast to (+)-propranolol (Rahn, Hawlina, Kersting & Planz, 1974), the antagonism of NA release does not seem to be necessary for the antihypertensive effect of these drugs.

This investigation was supported by a grant from INSERM (73.5.067.6).

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(Received March 15, 1979.)