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Effects of α- and β-adrenoceptor blockade on the neurally evoked overflow of endogenous noradrenaline from the rat isolated heart

A.M. Dart, R. Dietz, K. Hieronymus, W. Kübler, E. Mayer, A. Schömig & R. Strasser

Department of Cardiology, University of Heidelberg FRG.

1 β-Adrenoceptor blockade by propranol (4×10^{-6}M) was without effect on the overflow of endogenous noradrenaline from the isolated heart of the rat induced by stimulation of sympathetic nerves for 1 min at 1 and at 4Hz. The increase in heart rate in response to such stimulation was abolished by propranolol treatment.

2 α_2-Adrenoceptor blockade by yohimbine (10^{-6}M) induced approximately a two fold increase in the overflow of endogenous noradrenaline induced by stimulation of sympathetic nerves for 1 min at 1 and at 4 Hz.

3 A combination of yohimbine (10^{-6}M) and desipramine (10^{-7}M) induced a more than 3 fold increase in the overflow of endogenous noradrenaline produced by sympathetic nerve stimulation at 1 and at 4 Hz. Heart rate increases produced by such stimulation were intensified.

4 These results provide no evidence for the feedback stimulation of presynaptic β-adrenoceptors in this preparation. The action of α-$_2$-blockade was equipotent at stimulation frequencies of 1 and 4 Hz.

Introduction

The number of factors thought to participate in controlling the concentration of noradrenaline within the synaptic clefts of the peripheral sympathetic nervous system has grown appreciably in recent years. Thus in addition to nerve impulse frequency and uptake mechanisms, evidence has been presented for both α- (Starke *et al.*, 1975) and β- (Majewski *et al.*, 1981) adrenoceptor-mediated presynaptic control of noradrenaline release, although not all workers agree with the existence of a functional (α-mediated) autoinhibition (Kalsner, 1982). Evidence has been presented that presynaptic β-adrenoceptors can be stimulated by endogenously released noradrenaline thereby leading to a functional positive feedback (Adler-Graschinsky & Langer, 1975; Yamaguchi *et al.*, 1977). The subject of presynaptic control of noradrenaline release has recently been thoroughly reviewed (Langer, 1980). However, these control mechanisms have mostly been studied using radiolabelled (exogenously administered) noradrenaline, often in preparations lacking normal innervation.

We have recently described a preparation in which changes in the overflow of endogenous noradrenaline, induced by sympathetic nerve stimulation, from the otherwise isolated rat heart could be studied (Dart *et al.*, 1983). We have now studied the effects of the α-$_2$-antagonist yohimbine and of propranolol in this preparation.

Methods

These were essentially as described previously (Dart *et al.*, 1983). In brief, rats (150–200 g) were anaesthetized with thiobutabarbitone and prepared for *in situ* Langendorff perfusion with oxygenated modified Krebs-Henseleit solution (composition in mmol l^{-1}: Na^+ 144.14, K^+ 4.02, Ca^{2+} 1.85, Mg^{2+} 1.05, Cl^- 139.8, PO_4^{3-} 0.44, HCO_3^- 11.9, pyruvate 1.82, glucose 11.1, EDTA 0.0269).

Coronary venous effluent was collected via a cannula inserted through the inferior vena cava and ventricular contractions monitored with a force displacement transducer connected to a fine needle placed through the ventricular apex. The largest of the cardiac sympathetic nerves was then prepared for subsequent bipolar electrical stimulation (3V). Following collection of a prestimulation sample a 1 min stimulation was performed at 1 Hz, and after a 5 min interval, at 4 Hz. All coronary venous effluent was collected on ice, during stimulation and, separately,

during the first minute of the post stimulation period. This procedure was then repeated after a 15 min interval during which perfusion continued with Krebs-Henseleit solution containing yohimbine (10^{-6}M) (Serva), propranolol (4×10^{-6}M) (ICI-Pharma) or yohimbine (10^{-6}M) and desipramine (10^{-7}M) (Ciba-Geigy). Yohimbine and desipramine were dissolved in ethanol yielding final concentrations of 0.04% ethanol. Aliquots from each sample were mixed and added 1:1 to 0.6 N perchloric acid and samples were stored at − 80°C until subsequently assayed by a radioenzymatic assay (Da Prada & Zürcher, 1976) for noradrenaline. Yohimbine, desipramine and propranolol were without effect on the estimation of standard concentrations of noradrenaline. Statistical evaluation was performed with a paired *t*-test.

Results

In the absence of electrical stimulation only low levels of noradrenaline were detected in the coronary venous effluent and these were unaffected by the

Table 1 The effect of stimulation of a postganglionic cardiac sympathetic nerve on noradrenaline overflow and heart rate before (control) and after addition of yohimbine (10^{-6}M) to the perfusate ($n = 7$)

Sympathetic stimulation		*Noradrenaline overflow* (pmol per heart)	*Heart rate increase* (beat min^{-1})
Control	1 Hz	3.66± 1.77 (0.21±0.20)	16.8±25.2 (124.9±73.9)
	4 Hz	16.13± 7.46 (0.08±0.07)	31.0±27.0 (108.7±57.4)
Yohimbine (10^{-6}M)	1 Hz	*6.81± 2.53 (0.17±0.11)	12.7±21.3 (103.6±61.0)
	4 Hz	*33.60±15.20 (0.15±0.06)	28.0±27.2 (105.0±62.0)

Values in parentheses are the noradrenaline concentrations (pmol ml^{-1}) in the venous effluent and the heart rate (beats min^{-1}) in the minute immediately prior to each stimulation period. Stimulation was performed for one minute at both 1 and 4 impulses s^{-1}. Noradrenaline overflow was calculated from the noradrenaline recovered during stimulation and during the first minute post stimulation: heart rate increases relate to the minute of stimulation. *$P < 0.01$ (with respect to control values). Values given are mean ± s.d.

Table 2 The effect of stimulation of a postganglionic cardiac sympathetic nerve on noradrenaline overflow and heart rate before (control) and after addition of propranolol (4×10^{-6}M) to the perfusate ($n = 8$)

Sympathetic stimulation		*Noradrenaline overflow* (pmol per heart)	*Heart rate increase* (beats min^{-1})
Control	1 Hz	4.37±1.33 (0.30±0.15)	7.6±11.3 (100.4±55.6)
	4 Hz	13.44±6.11 (0.28±0.12)	21.0±10.0 (100.1±51.1)
Propranolol	1 Hz	4.82±1.98 (0.32±0.16)	−1.0± 7.7 (105.3±34.8)
(4×10^{-6}M)	4 Hz	16.94±9.60 (0.32±0.17)	*1.6± 6.1 (103.6±30.3)

See Table 1 for further details.

Table 3 The effect of stimulation of a postganglionic cardiac sympathetic nerve on noradrenaline overflow and heart rate before (control) and after addition of yohimbine (10^{-6}M) and desipramine (10^{-7}M) to the perfusate ($n = 8$)

Sympathetic stimulation		*Noradrenaline overflow* (pmol per heart)	*Heart rate increase* (beats min^{-1})
Control	1 Hz	3.13± 0.86 (0.11±0.08)	13.7±13.3 (141.5±48.1)
	4 HZ	10.76 2.60 (0.21±0.15)	22.5±13.6 (143.3±42.8)
Yohimbine (10^{-6}M)	1 Hz	*9.74± 4.16 (0.16±0.09)	**26.7±12.8 (121.1±38.3)
Desipramine (10^{-7}M)	4 Hz	*38.30±13.10 (0.19±0.11)	40.8±16.9 (119.8±47.2)

** $P < 0.05$.
See Table 1 for further details.

addition of yohimbine, propranolol or yohimbine and desipramine (see figures in parentheses, Tables 1, 2, 3). Pre-stimulation resting heart rates were also unaffected by these agents with the exception of the combination of yohimbine and desipramine which produced a reduction ($P < 0.05$) in heart rate in the 1 Hz series (see Tables 1, 2 and 3).

The effects of yohimbine (10^{-6}M) on the noradrenaline overflow and heart rate increase produced by sympathetic nerve stimulation are shown in Table 1. Yohimbine produced significant ($P < 0.01$) increases in noradrenaline overflow in response to nerve stimulation at both 1 and 4 Hz. The heart rate increases produced by such stimulation were somewhat lower in the presence of yohimbine although the reduction was not significant at the $P = 0.05$ level. Propranolol (4×10^{-6}M) abolished the increase in heart rate produced by nerve stimulation but was without effect on the noradrenaline overflow produced by such stimulation (Table 2). The combination of desipramine (10^{-7}M) and yohimbine (10^{-6}M) produced further increase in the overflow of noradrenaline (Table 3). In addition, the combination produced increases in the heart rate responses (at 1 and 4 Hz) produced by sympathetic nerve stimulation, but these failed to reach statistical significance.

Discussion

These findings demonstrate a potent effect of yohimbine on the neurally evoked overflow of endogenous noradrenaline, both in the presence and absence of neuronal uptake blockade. Yohimbine administration produced no significant changes in the heart rate responses induced by sympathetic nerve stimulation. A concentration of 10^{-6}M was used in these experiments since such a concentration has been found to be nearly maximal in increasing the overflow of tritiated noradrenaline from stimulated pulmonary artery strips without any clear toxic effect (Starke *et al.*, 1975). In this preparation yohimbine (10^{-6}M) showed no significant effect on basal heart rates. However, the effect of a combination of desipramine and yohimbine on heart rate increase may have been underestimated since this combination did produce a significant reduction in resting heart rate (in the 1 Hz series), perhaps indicating a toxic action on effector cells. Although the sympathetically mediated increases in heart rate found in this study were lower and less uniform than those found previously (Dart *et al.*, 1983) the relative relationship between the effects of stimulation at 1 and 4 Hz was maintained.

The effect of yohimbine on noradrenaline overflow was clearly demonstrable at stimulation frequencies of both 1 and 4 Hz. Single fibre recordings from cardiac sympathetic efferent nerves in the cat (Kollai & Koizumi, 1980) have demonstrated a mean firing frequency of 1.8 impulses s^{-1} so that this presynaptic effect of yohimbine is probably present at both basal as well as augmented levels of sympathetic activity. The *in vivo* augmented firing frequency in the cardiac efferent nerves in the rat is unknown but effects of sympathetic vasoconstrictor stimulation in the cat hindlimb are almost maximal (80–85%) at a frequency of 6 impulses s^{-1} (Folkow, 1952) and single unit recordings in the cat show similar firing frequency for cardiac sympathetic and fore-limb vasoconstrictor nerves (Kollai & Koizumi, 1980). The functional significance of an autoinhibition that operates to a similar degree at both augmented and basal levels of neural activity is not clear since such a system would not seem to be capable of selectively diminishing the basal release of noradrenaline and thereby amplifying any signal.

Propranolol failed to influence the overflow of noradrenaline produced by nerve stimulation at either 1 or 4 Hz. The heart rate increase produced by such stimulation was, as expected, abolished by propranolol administration indicating effective blockade of post-synaptic β-adrenoceptors. These experiments therefore provide no evidence to support the concept of a functionally significant feedback stimulation of presynaptic β-adrenoceptors (Adler-Graschinsky & Langer, 1975; Yamaguchi *et al.*, 1977). Our results do not exclude the possibility that such receptors could be stimulated by adrenaline.

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