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Effects of cocaine and desipramine on the neurally evoked overflow of endogenous noradrenaline from the rat heart

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1 Stimulation of postganglionic cardiac sympathetic nerves produced a stimulation frequency-dependent overflow of endogenous noradrenaline from the otherwise isolated rat heart.

2 Such nerve stimulation also produced increases in heart rate. There was significant correlation between heart rate increases and corresponding noradrenaline concentrations in the coronary venous effluent.

3 Cocaine (3×10^{-5} M) caused a significant reduction in both the noradrenaline overflow and the heart rate increase, produced by nerve stimulation for 1 min at 4 Hz.

4 Desipramine (10^{-6} M) caused a significant increase in the noradrenaline overflow produced by stimulation for 1 min (4 Hz) with a mean increase of approximately 60%. There was no significant effect on the heart rate increase produced by such stimulation.

5 The opposite effects of cocaine (3×10^{-5} M) and desipramine (10^{-6} M) on noradrenaline overflow are attributed to differences in the local anaesthetic properties of these agents.

Introduction

The major mechanism for the removal of noradrenaline released into the synaptic clefts of the peripheral sympathetic nervous system is generally thought to be by re-uptake into the nerve terminals. Studies of the myocardial metabolism of noradrenaline frequently employ cocaine in order to block this uptake process. Complete blockade of the uptake of radiolabelled noradrenaline in the isolated perfused heart of the rat is only approached at concentrations of at least 3×10^{-5} M (Iversen, 1963) and concentrations of 3×10^{-5} M are therefore frequently used in studies of myocardial noradrenaline metabolism (Dietz, Schömig, Strasser & Kübler, 1981). However, the effect of this concentration of cocaine on the overflow of endogenous noradrenaline induced by sympathetic nerve activity from the otherwise isolated heart is unknown. We have therefore investigated the action of cocaine under these conditions and compared it with those of another agent, desipramine, which is also known, at concentrations of 10^{-6} M, to block completely the neuronal uptake of radiolabelled noradrenaline (Titus & Spiegel, 1962) but which differs from cocaine in having no local anaesthetic activity.

Methods

The study was performed on Wistar rats weighing 150–200 g. They were anaesthetized with thiobutabarbitone (50 mg/kg i.p.), the thorax was opened and a metal cannula inserted and tied into the ascending aorta for retrograde perfusion (Langendorff technique). A polythene cannula was introduced into the inferior vena cava and advanced to lie just within the heart for collection of the coronary venous effluent. The hearts were perfused with oxygenated modified Krebs-Henseleit solution (Dietz *et al.*, 1981) at a rate of 3.5 ml g^{-1} min^{-1}. The pH of the perfusate was adjusted to 7.4 with CO_2 and the temperature adjusted to lie within the range 35–37°C at the point of entry into the aorta.

It was found unnecessary to ligate blood vessels other than the inferior vena cava since in most experiments perfusate recovery was almost complete without any further procedures. In all experiments the completeness of perfusate recovery was determined at the beginning and end of each experiment: experiments in which recovery was less than 85% were discarded.

A thin (6–0) curved needle was placed through the apex of the heart and attached with fine thread to a Grass force- displacement transducer for subsequent monitoring of ventricular contractions. The left cervico-thoracic ganglion was then exposed under a dissecting microscope and the largest of the exiting (postganglionic) cardiac nerves dissected free for subsequent electrical stimulation. The ganglion was tied with a thread close to the exit of the chosen nerve so that subsequent manipulation of the nerve could be performed without direct handling. The nerve and adjacent ganglion were then isolated from the remainder of the sympathetic chain. When the nerve was not being stimulated it was superperfused with warmed oxygenated Krebs-Henseleit solution. Bipolar steel electrodes, held in a micromanipulator, were used for electrical stimulation. The electrode closest to the heart was always the cathode and stimulation was performed with monophasic square pulses of 2 ms duration and 3V intensity.

In an initial series of experiments the effects of changes in stimulation frequency on both noradrenaline overflow and heart rate were determined. The freed nerve was first laid over the stimulating electrodes and a pre-stimulation sample collected. Stimulation was then performed for 1 min at various frequencies (1, 4, 8, 12 and 20 Hz). The venous effluent was collected throughout the period of stimulation and the heart rate monitored before and during stimulation. Heart rate increases were subsequently determined by comparing the total number of ventricular contractions occurring during each minute of stimulation with the number in the respective prestimulation period. The order of stimulation frequencies was varied to minimize errors arising from transmitter depletion.

The effects of desipramine (10^{-6} M) and cocaine (3×10^{-5} M) were studied in a second series of experiments in which stimulation was performed at 4 Hz. After placement of the nerve on the electrodes, and collection of a prestimulation sample, stimulation was performed for 1 min at 4 Hz and the venous effluent collected during this time. The hearts were then perfused with either Krebs-Henseleit solution, Krebs-Henseleit solution containing cocaine (3×10^{-5} M) or Krebs-Henseleit solution containing desipramine (10^{-6} M in 0.04% ethanol). Stimulation (at 4 Hz) was then repeated after an interval of 15 min. Heart rate (ventricular contractions) was monitored before and during each stimulation period. Samples were collected onto ice and immediately mixed; 250 µl aliquots were then added to 250 µl of previously chilled 0.6 N perchloric acid. Samples were stored at −80°C until assayed. Samples were assayed, without prior concentration, for noradrenaline, adrenaline and dopamine by a radioenzymatic assay (Da Prada & Zürcher, 1976). Standard concentrations of noradrenaline (in Krebs-Henseleit solution) were also assayed in the presence and absence of cocaine (3×10^{-5} M) and desipramine (10^{-6} M).

Krebs-Henseleit solution, without noradrenaline, yielded 25.1 ct/min in the presence of cocaine, 28.6 ct/min in the presence of desipramine and 34.9 ct/min in the absence of these agents. Krebs-Henseleit solutions containing noradrenaline yielded 29.2 ct min^{-1} pg^{-1} noradrenaline in the presence of cocaine, 28.1 ct min^{-1} pg^{-1} in the presence of desipramine and 30.3 ct min^{-1} pg^{-1} in the absence of these agents. The intra-assay coefficient of variation was less than 5%. The paired t test was used for statistical evaluation of the effects of intervention with cocaine and desipramine; the Kruskal-Wallis test for evaluation of the effects of change in stimulation frequency.

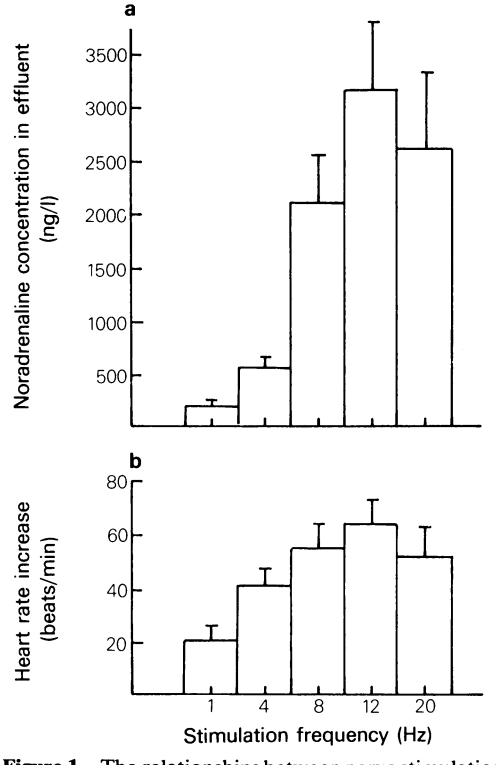

Figure 1 The relationships between nerve stimulation-frequency and resulting noradrenaline concentration in the venous effluent (a) and heart rate increase (b) are shown. All stimulation periods were of 1 min duration. Values are means of $n = 10$; vertical lines indicate s.e.mean. For statistical evaluation see text.

Results

In the absence of electrical stimulation no adrenaline or dopamine was detected in the coronary venous effluent. Noradrenaline was either not detected or was present at concentrations of less than 45 ng/l. During electrical stimulation there was an overflow of noradrenaline and of adrenaline (at approximately 5% of the noradrenaline output) but not of dopamine. The overflow of catecholamines varied considerably from rat to rat and this variation was dependent on the particular anatomical arrangement (i.e. whether there were one or two large nerves or several smaller ones).

The relationships between nerve stimulation-frequency and both noradrenaline overflow and heart rate increase are shown in Figure 1. There were significant differences, with respect to noradrenaline overflow, between stimulation at 1 and 4 ($P < 0.05$) and between 4 and 8 Hz ($P < 0.01$). The differences between 8, 12 and 20 Hz were not significant. Heart rate responses showed significant differences between stimulation at 1 and 4 ($P < 0.05$) and between 4 and 12 Hz ($P < 0.01$) but other differences were not statistically significant. Calculation of the regression coefficient between heart rate increase and log noradrenaline concentration showed a highly significant correlation ($P < 0.001$) with $r = 0.515$.

Table 1 The overflow of endogenous noradrenaline induced by stimulation (4 Hz) for 1 min of a postganglionic sympathetic nerve before (S1) and after (S2) a 15 min interval during which perfusion continued with Krebs-Henseleit solution, alone (control), with cocaine (3×10^{-5} M) or with desipramine (10^{-6} M)

	Noradrenaline concentration in effluent (ng/l)	
	Stimulation (S1)	*Stimulation (S2)*
Control	401	384
($n = 8$)	419	393
	85	86
	181	250
	946	1176
	405	344
	657	891
	159	132
Mean	406 ± 101	457 ± 134
Desipramine	449	394
(10^{-6} M)	156	330
($n = 7$)	427	1046
	159	576
	836	1440
	404	504
	958	1026
Mean	484 ± 117	759 ± 156*
Cocaine	1363	185
(3×10^{-5} M)	231	221
($n = 9$)	1158	273
	115	182
	591	16
	442	29
	564	0
	305	115
	326	52
Mean	566 ± 142	119 ± 100*

* $P < 0.05$, paired t test

Each pair of results represents a separate experiment. Individual values and the means (± s.e.mean) for each group are given.

The results of the second series of experiments are shown in Table 1. In the control series there was no significant difference between the noradrenaline concentrations found during the first (S1) and second (S2) stimulations. However, intervention with desipramine caused a significant increase ($P < 0.05$) and with cocaine a significant reduction ($P < 0.05$) in the noradrenaline concentration during the second period of stimulation.

Cocaine also caused a significant ($P < 0.01$) reduction in the heart rate increase produced by sympathetic nerve stimulation (S1 + 44 ± 11, S2 + 15 ± 7 beats/min). However, in the control series, and following intervention with desipramine, there was no significant difference between the heart rate increases produced by S1 and S2 (Figure 2).

Discussion

Previous studies of the importance of the neuronal reuptake process for the clearance of noradrenaline from the sympathetic synaptic cleft have largely used radiolabelled noradrenaline with the assumption that the behaviour of this reflects accurately that of the endogenous catecholamine. In addition, studies of myocardial noradrenaline metabolism have usually employed isolated organs without functional nerve innervation. The estimation of neurally-induced endogenous noradrenaline overflow from the otherwise isolated heart allows a more physiological approach to problems of myocardial noradrenaline release and metabolism.

Electrical stimulation of postganglionic sympathetic cardiac nerves resulted in a frequency-dependent overflow of noradrenaline into the venous effluent. Such stimulation also produced an increase in heart rate. The relationship between increase in heart rate and stimulation frequency was much flatter over the range 1–8 Hz than the relationship between noradrenaline overfow and stimulation frequency.

Cocaine, at the concentration (3×10^{-5} M) which has previously been used to block completely the uptake of labelled noradrenaline in the perfused rat

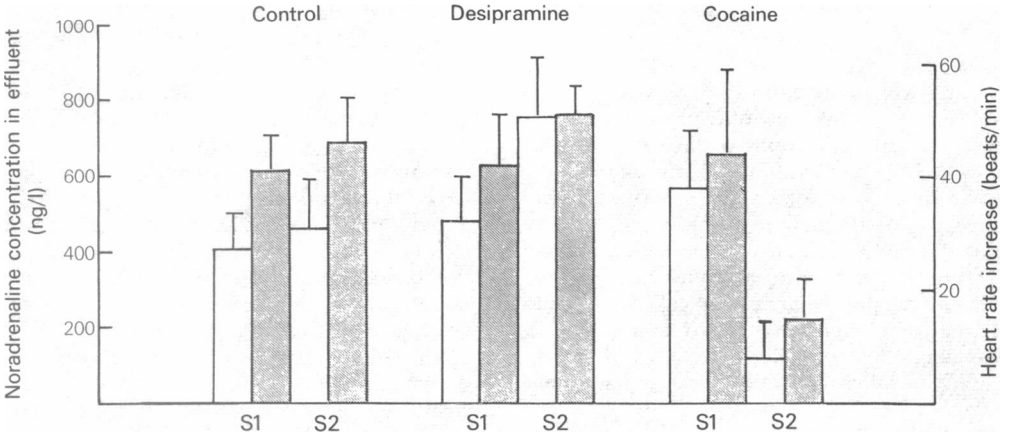

Figure 2 Noradrenaline concentrations in the coronary venous effluent (open columns) and corresponding heart rate increases (hatched columns) produced by sympathetic stimulation (4 Hz) for 1 min are shown. S1 values were obtained before, and S2 values after, a 15 min interval during which perfusion continued with Krebs-Henseleit solution alone (control, $n = 8$), with Krebs-Henseleit solution containing cocaine (3×10^{-5} M, $n = 9$) or with Krebs-Henseleit solution containing desipramine (10^{-6} M, $n = 7$). Values are means; vertical lines indicate s.e.mean. Differences were statistically significant between S1 and S2 noradrenaline concentrations in the desipramine ($P < 0.05$) and cocaine ($P < 0.05$) series, and between S1 and S2 heart rate changes in the cocaine series ($P < 0.01$).

heart (Dietz *et al.*, 1981), caused a significant reduction in the overflow of noradrenaline. In addition, there was a significant reduction in the heart rate increase produced by sympathetic nerve stimulation. It seems likely that in this preparation cocaine, at 3×10^{-5} M, acts as a local anaesthetic to the nerve fibres during their intracardiac course. Since lower concentrations of cocaine fail to block the neuronal uptake of noradrenaline completely it is clearly an unsuitable agent to use in this preparation.

Blockade of neuronal uptake by an agent without local anaesthetic properties, desipramine, caused an increase in noradrenaline overflow of approximately 60% during nerve stimulation at 4 Hz. However, this may be an unduly low assessment of the usual importance of neuronal uptake since the operation of other homeostatic mechanisms (extraneuronal uptake, presynaptic inhibition) may attenuate the effects of neuronal uptake blockade. This enhanced noradrenaline overflow was not accompanied by a significant change in the sympathetically mediated increase in heart rate. However such a change might not be expected in this model since increase of the stimulation frequency from 4 to 8 Hz produced no further significant increase in heart rate response (despite an almost four fold increase in the overflow of noradrenaline).

The differing effects of cocaine and desipramine have demonstrated the ability of this experimental model to permit the detection of increases and decreases in the neurally induced overflow of endogenous catecholamines from the otherwise isolated heart of the rat.

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References

DA PRADA, M. & ZÜRCHER, G. (1976). Simultaneous radioenzymatic determination of plasma and tissue adrenaline, noradrenaline and dopamine within fentomole range. *Life Sci.*, **19**, 1161–1174.

DIETZ, R., SCHÖMIG, A., STRASSER, R. & KÜBLER, W. (1981). Catecholamines in myocardial hypoxia and ischaemia. In *Catecholamines and the Heart.* ed., Delius, W., Gerlach, E., Grobecker, H. & Kübler, W. pp. 201–208. Berlin, Heidelberg, New York: Springer Verlag.

IVERSEN, L.L. (1963). The uptake of noradrenaline by the isolated perfused rat heart. *Br. J. Pharmac. Chemothar.*, **21**, 523–537.

TITUS, E.O. & SPIEGEL, H.E. (1962). Effect of desmethylimipramine (DMI) on uptake of noradrenaline-7-H3 (NE) in heart. *Fed. Proc.*, **21**, 179.

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