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NORADRENALINE RELEASE IN RATS DURING PROLONGED COLD-STRESS AND REPEATED SWIM-STRESS

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1 Plasma noradrenaline concentration in rats was measured during prolonged cold-stress and repeated swim-stress.

2 Cold exposure for 6 h caused a rise in plasma noradrenaline which reached a peak at 4 h.

3 Administration of desmethylimipramine and normetanephrine to block neuronal and extraneuronal uptake of noradrenaline raised plasma noradrenaline concentration without changing the pattern of the response to cold exposure.

4 Repeated cold exposure on subsequent days produced no change in the pattern of plasma noradrenaline concentration.

5 Five successive 1-min swims at 30-min intervals caused a rise in plasma noradrenaline concentration which was maximal after the third swim.

6 It is suggested that prolonged and repeated activation of sympathetic nerve terminals leads to a decline in noradrenaline release.

Introduction

The suggestion that cold exposure causes increased activation of the peripheral sympathetic nervous system rests on two sources of evidence: an increase in the turnover rate of noradrenaline in organs with a sympathetic innervation (Spector, Gordon, Sjoerdsma & Udenfriend, 1967; Costa, Neff & Ngai, 1969; Bralet, Beley & Lallemant, 1972) and an increase in the noradrenaline excretion in the urine (Leduc, 1961; Shum, Johnson & Flattery, 1969; Bibbiani & Viola-Magni, 1971; Konzett, Hörtnagl, Hörtnagl & Winkler, 1971).

The demonstration that nerve terminals released noradrenaline (von Euler, 1946) and that noradrenaline was present in the urine (von Euler & Hellner, 1951) raised the possibility that plasma noradrenaline might provide a measure of the activity of the sympathetic nervous system. However, of the noradrenaline released from sympathetic nerve terminals, only that fraction which has escaped neuronal and extraneuronal uptake enters the blood stream. These uptake processes, together with renal excretion, are also responsible for removal of noradrenaline from the circulation. Noradrenaline in the blood stream therefore has only a short half-life which has been estimated at 1.5 ~ 2 min in the rat (Vane, 1969; Benedict, Fillenz, Stanford & Valero, 1977). Although the level of plasma noradrenaline is the outcome of these and other interacting processes, we have shown that it can be used as an index of noradrenaline release rate (Benedict, Fillenz & Stanford, 1978). The aim of the present study was to examine and compare the changes in release rate during prolonged, steady and repeated brief activation of the sympathetic nervous system. To achieve these different levels of activity we have used cold exposure and repeated, brief swim-stress.

A preliminary communication of some of these results has been published (Benedict, Fillenz & Stanford, 1977).

Methods

Male Sprague–Dawley rats of 200 to 350 g body weight were used. Intra-atrial cannulae were inserted through the jugular vein as described in a previous paper (Benedict, *et al.*, 1978). Samples of 0.25 ml blood were withdrawn through the cannula and replaced with an equal volume of sterile 0.9% w/v NaCl solution (saline). The noradrenaline content of the plasma was measured by a radioenzymatic method (Hörtnagl, Benedict, Grahame-Smith & McGrath, 1977).

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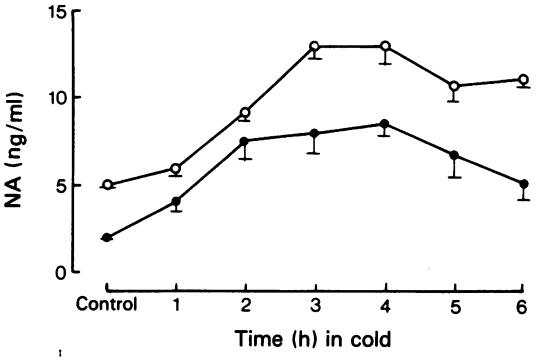

Figure 1 Effect of desmethylimipramine (DMI) and normetanephrine (NMN) on plasma noradrenaline (NA) levels during cold-stress in rats. DMI 50 mg/kg and NMN 10 mg/kg were injected (i.p.) 30 min before the onset of stress. $n = 6$ for cold alone (●); $n = 4$ for cold + DMI + NMN (○). Error bars are s.e. mean.

Drugs

The following drugs were used: normetanephrine (NMN; Sigma) 10 mg/kg intraperitoneally; desmethylimipramine, DMI (Pertofran; Geigy) 50 mg/kg intraperitoneally.

Results

Plasma noradrenaline was measured at hourly intervals in rats kept in a cold room at 4°C. Figure 1 shows that on cold exposure, plasma noradrenaline rose rapidly in the first 2 h; this was followed by a slower rise in the next 2 h. After reaching a peak level at 4 h, noradrenaline concentration in the plasma started to fall. In order to see whether the variation in plasma noradrenaline concentration reflected changes in either the rate of noradrenaline release, and therefore of entry into the circulation, or of noradrenaline removal, the experiment was repeated with rats pretreated with normetanephrine (NMN) and desmethylimipramine (DMI), in a single intraperitoneal injection.

In the presence of these two drugs, which block neuronal and extraneuronal uptake of noradrenaline, plasma noradrenaline concentrations were higher than those in untreated rats, both before and throughout cold exposure. However, the changes during cold exposure followed the same pattern as they did in the untreated rats; there was a peak of plasma noradrenaline which was not maintained (Figure 1).

A number of rats were exposed to a second cold-stress at intervals of 24, 48 and 72 h after the first exposure. The results (Figure 2) showed no difference in response between the first and second cold stress at any of these intervals. In one rat, plasma noradrenaline concentration was measured during four successive cold exposures at intervals of 3, 4 and 7 days after the initial stress. Again, the general pattern in plasma noradrenaline concentration remained the same throughout successive cold exposures.

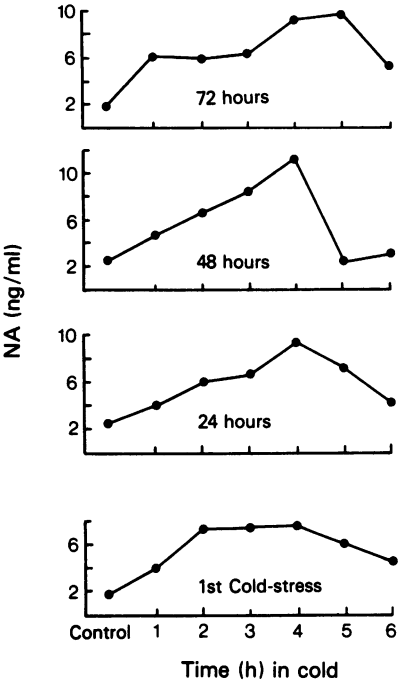

Figure 2 Changes in plasma noradrenaline (NA) concentrations during an initial and during a subsequent cold exposure 24, 48 or 72 h after the first one. $n = 5$ for 1st cold stress, $n = 2$ for 24 h, $n = 2$ for 48 h and $n = 1$ for 72 h.

In order to compare the effect of a continued stimulus to the sympathetic nervous system with brief stimulation, rats were given a 1-min swim-stress in water at 10°C., repeated at 30-min intervals. Plasma samples were taken at the end of each swim while the animals were still in the water. Figure 3 shows that the first three swims led to progressively greater increases in plasma noradrenaline, after which the response showed a sharp decline.

Discussion

We have shown in a previous paper that although plasma noradrenaline concentration is the outcome of a number of interacting mechanisms, changes in plasma noradrenaline can be used to monitor changes in noradrenaline release rate, (Benedict *et al.*, 1978). Six hours cold exposure causes a rise, followed by a fall, in plasma noradrenaline concentration. The rising plasma noradrenaline concentration could

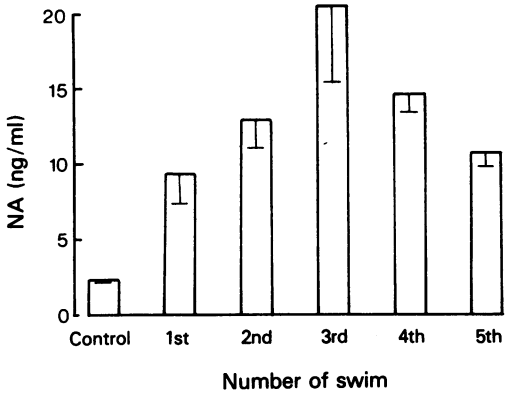

Figure 3 Noradrenaline (NA) concentration of plasma samples taken at the end of five successive 1-min swims. Water temp. 10°C; interval between swims 30 min; height of column is mean; vertical bars are s.e. mean. $n = 3$.

result from either a rising release rate or a constant release rate which exceeds the rate of removal, thus leading to noradrenaline accumulation. The experiments after pretreatment with DMI and NMN which affect removal, but not release of noradrenaline, show that the rate of the increase in noradrenaline concentration in plasma is not affected by NMN and DMI. This suggests that the rise represents increasing release rate rather than accumulation of noradrenaline. The same is suggested by the swim-stress experiments. Noradrenaline released by swim-stress is rapidly removed from the plasma (Benedict, *et al.*, 1978). Even in the presence of DMI and NMN there is an initial rate of removal giving a half-life of only 0.4 s. There is a second slower phase of noradrenaline removal, but plasma noradrenaline levels return to normal by 30 min. Between each swim, therefore, plasma noradrenaline is restored to control levels, eliminating the possibility that the progressive rise in noradrenaline concentration with successive swims represents accumulation of noradrenaline. The results thus favour the possibility of greater release rate.

The fall in plasma noradrenaline after 4 h cold exposure and with repeated swim-stress suggests a progressive failure of the transmitter release mechanisms. This seems to occur both with continued activation, as in the cold exposure, and repeated, brief, but intense stimulation, as with the swim-stress. Whatever the nature of the failure of release, rest leads to recovery since as little as 18 h after the end of the first cold exposure (the earliest time interval tested), a second cold exposure produces a response in terms of plasma noradrenaline changes which is the same as the first. It has been shown that cold stress leads to induction of tyrosine hydroxylase and dopamine-β-hydroxylase (Thoenen, 1970). The increase in the amount of enzyme occurs with a delay of 18 to 24 h in the cell body and would be expected to reach the terminal after the additional delay involved in axoplasmic transport. There is some evidence to suggest that this takes 7 days (Thoenen, 1970). In the present experiments there was no evidence that enzyme induction had any effect on noradrenaline release since the rise in plasma noradrenaline was not increased at this time and there was still a fall after 4 h.

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