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CHANGES IN PLASMA NORADRENALINE CONCENTRATION AS A MEASURE OF RELEASE RATE

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1 A method is described for repeated sampling of plasma noradrenaline (NA) in freely moving rats. Na concentration does not change during the day or after adrenalectomy.

2 Exogenous NA has a half-life of 1.5 min; drugs which block neuronal and extra-neuronal uptake lengthen this to 6.3 min.

3 Swim-stress leads to a steep rise followed by a rapid decline in plasma NA concentration.

4 This method of plasma NA sampling can serve as a measure of both steady and rapid changes in release rate over long periods of time.

Introduction

Noradrenaline is the only neurotransmitter which appears in significant concentrations in the blood stream. It is stored both in sympathetic neurones and in adrenal medullary cells. There is no extracellular enzyme for its metabolism, but there are active transport processes for its uptake into neurones and non-neuronal cells. Its concentration in the blood therefore is the outcome of the interaction of a number of different processes.

The present experiments were undertaken to see whether plasma noradrenaline concentrations in the rat could serve as a measure of noradrenaline release and could be used to monitor transmitter release *in vivo* over long periods of time.

Methods

Male Sprague-Dawley rats weighing 200–300 g were used. Under Hypnorm anaesthesia, a cannula was tied into the superior vena cava with its tip in the right atrium. Polythene tubing connected to the cannula was led under the skin to emerge at the surface in the midscapular region. A minimum of 24 h was allowed for recovery after the operation. Samples of blood of 0.25 ml were withdrawn and replaced with an equal volume of sterile 0.9% w/v NaCl solution (saline). The blood was put into tubes containing 0.4 mg glutathione per ml of blood and 35 µl heparin. After centrifugation the plasma was diluted in 4 volumes of distilled water and stored at −10°C. Exogenous noradrenaline was administered via the intra-atrial cannula. Some of the rats were subjected to a 1 min swim-stress by placing them in a bucket of water. Bilateral adrenalectomy under Hypnorm anaesthesia was carried out by a dorsal approach. The minimum period of recovery after surgery was 24 h. These animals were provided with saline for their drinking water. The noradrenaline concentration in the plasma samples was assayed by a radiochemical enzymatic method (Hörtnagl, Benedict, Grahame-Smith & McGrath, 1977).

Drugs

The following drugs were used: (−)-noradrenaline (free base) which was dissolved in saline for intravenous injection; desmethylimipramine ('Pertofran'; Geigy); normetanephrine (Sigma) and Hypnorm (Janssen).

Results

The plasma noradrenaline concentration in control rats was 1.88 ± 0.08 ng/ml (n = 16) with a coefficient of variation of 17 o/o. In order to see whether plasma noradrenaline varied during the day, hourly samples were assayed in three rats between 11 h 00 min and 17 h 00 min. Table 1 shows the mean noradrenaline concentration with the s.e. mean and the coefficient of variation for each rat over the 6 h period.

Since released noradrenaline is subject to neuronal

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and extra-neuronal uptake, desmethylimipramine (DMI) 10 mg/kg and normetanephrine (NMN) 50 mg/kg were given in a single intraperitoneal injection to block the two uptake processes. Half an hour after the injection, plasma noradrenaline had risen to 5.26 ± 0.51 ng/ml (n = 9).

In order to assess the relative contributions of the adrenals and the sympathetic nerve terminals to plasma noradrenaline, one group of rats was adrenalectomized at the time of cannulation; 24 h after adrenalectomy, plasma noradrenaline was 1.59 ± 0.13 ng/ml (n = 5); this is not significantly different from the value in control rats.

To study the removal of noradrenaline from the blood stream, 100 μg/kg noradrenaline was injected through the atrial cannula. The changes in plasma noradrenaline with time are shown in Figure 1a. The characteristics of the early, rapid part of the decay were examined by repeating the experiment with a group of previously untreated rats and comparing the results with those from a group pretreated with DMI and NMN in a single injection 30 min before administration of exogenous noradrenaline. Figure 2(a and b) shows the plasma noradrenaline concentrations for these two groups of rats on semi-logarithmic coordinates.

The plasma noradrenaline concentration 1 min after injection was 103.12 ± 2.24 ng/ml in the untreated rats and 1093.8 ± 6.33 ng/ml in the rats pretreated with DMI and NMN. The straight line in Figure 2a is the regression line calculated from the figures for noradrenaline concentration from 1 to 3 min after which noradrenaline decays more slowly. The half-life for the exponential decline in NA concentration in Figure 2a is 1.5 min while in rats pretreated with DMI and NMN the half-life for noradrenaline disappearance was 6.3 min.

In order to compare the fate of endogenously released with exogenous noradrenaline, rats were subjected to a 1 min swim-stress. The changes in plasma noradrenaline concentration are shown in Figure 1b. The early decline of this endogenous noradrenaline was measured in two groups of rats, the first given a 1 min swim-stress only, and the second pretreated with DMI and NMN before the swim. The results, plotted semi-logarithmically, are shown in Figure 2(c and d).

In the first group of animals, the plasma noradrenaline concentration in a sample taken at the end of 1 min swim-stress, but while the animals were still in the water (water temp. 10°C) was 45.28 ± 1.51 ng/ml (n = 3). In the first minute after swim-stress, plasma noradrenaline fell to 39.96 ± 1.35 ng/ml and in the second minute to 12.68 ± 0.36 ng/ml. In the presence of DMI and NMN swim-stress caused a rise in plasma noradrenaline to 299.59 ± 2.34 ng/ml after which it fell steeply during the first 2 min. The regression line fitted to the data for the first 2 min corresponds to a half-life of 0.4 min. After this, plasma noradrenaline declined more slowly.

Discussion

Since von Euler and Hellner's demonstration in 1951 that catecholamines were present in the urine, attempts have been made to measure catecholamine levels in the blood. Early values for rat plasma catecholamine were much higher and more variable than those for man. That this was due to stress accompanying sample collection is evident by the progressive lowering of values for plasma catecholamines with improvements of sample collection and assay. Since the review by Callingham (1975) a number of papers have appeared giving a wide range of values for plasma noradrenaline in the rat. Depocas & Behrens (1977) have measured the rise in plasma noradrenaline caused by handling, anaesthesia, surgery and

Table 1 Variation of plasma noradrenaline concentration with time of day

Plasma NA (ng/ml)	*Time of day (hours)* 11.00	12.00	13.00	14.00	15.00	16.00	17.00	Mean ± s.e. mean	Cv
Rat 1	2.37	2.28	2.37	2.84	2.31	2.01	2.63	2.26 ± 0.13	16.4
Rat 2	2.28	2.36	1.82	2.34	2.93	2.07	2.47	2.32 ± 0.13	14.8
Rat 3	2.53	2.66	2.63	2.42	2.18	2.32	1.96	2.38 ± 0.09	10.6
Mean	2.39	2.43	2.27	2.53	2.47	2.13	2.02		
± s.e. mean	±0.07	±0.11	±0.24	±0.15	±0.23	±0.09	±0.24		
Cv	5.2	8.2	18.2	10.6	16.2	7.7	20.9		

Values shown for each sample taken from 3 rats at hourly intervals. Mean, s.e. mean and coefficient of variation (Cv) shown for each rat with values pooled over 6 h period and each time with pooled values for 3 animals.

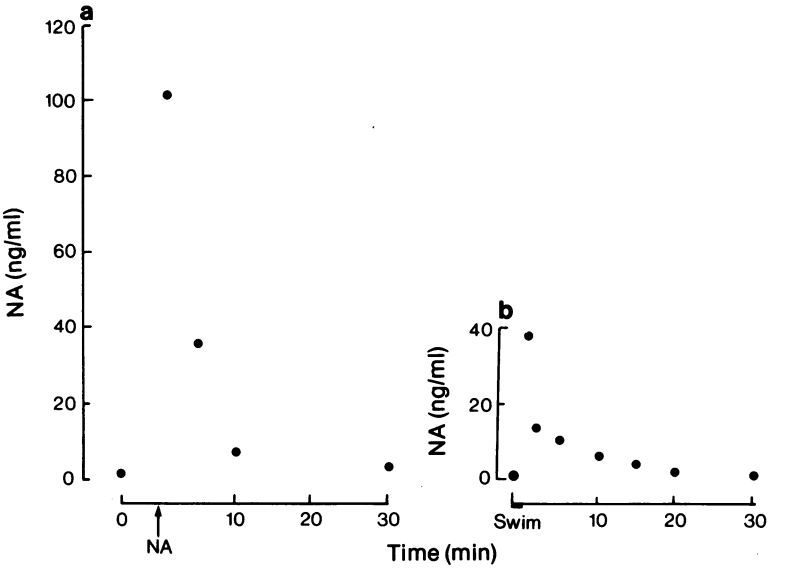

Figure 1 Noradrenaline (NA) concentration in rats expressed as ng/ml plasma. (a) Plasma NA levels before and after a pulse injection of exogenous NA (100 μg/kg); (b) plasma NA levels before and after a 1 min swim-stress.

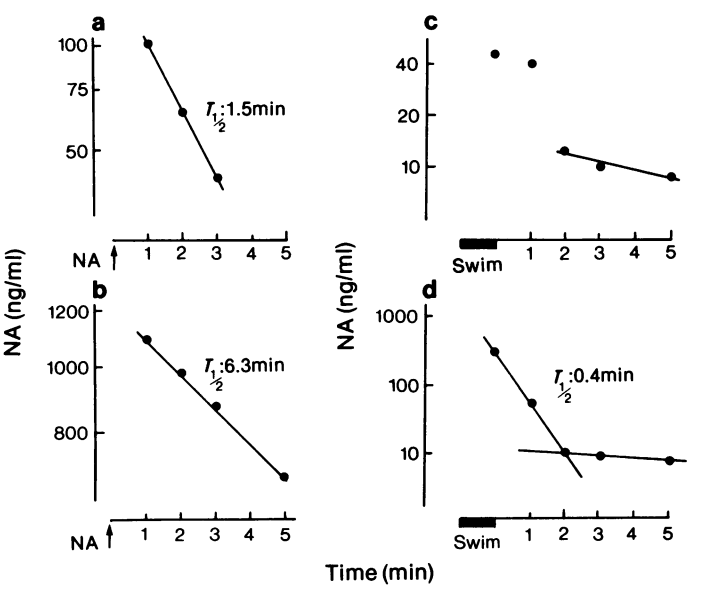

Figure 2 Rate of disappearance of noradrenaline (NA) from plasma of rats. Abscissae: NA concentration expressed as ng/ml plasma, plotted logarithmically. Ordinates: time in minutes. Rate of removal shown after: (a) A single injection of exogenous NA (100 μg/kg); (b) a single injection of exogenous NA in animals pre-treated with desmethylimipramine (DMI, 10 mg/kg) plus normetanephrine (NMN, 50 mg/kg); (c) 1 min swim-stress; (d) 1 min swim-stress of animals pretreated with DMI (10 mg/kg) plus NMN (50 mg/kg).

decapitation. Blood collected from indwelling cannulae in rats kept in metabolic chambers, warm acclimatised and fed on a semi-synthetic diet had a noradrenaline concentration of 0.15 ng/ml of plasma; this is the lowest figure reported to date. The concentration of 1.6 ng/ml found in the present study is in close agreement with that of a number of publications where the environment of the animals was less strictly controlled than in the study by Depocas & Behrens (Reid, Dargie, Franklin & Fraser, 1976; Nagaoka & Lovenberg, 1976; Grobecker, Roizen & Kopin, 1977; Popper, Chiueh & Kopin, 1977).

The origin of plasma noradrenaline has been studied in experiments in which attempts were made to eliminate one of the two possible sources; however, the results of these experiments have not provided a conclusive answer to the problem. Although adrenal medullary cells store and release noradrenaline, adrenalectomy causes either no change, as in the present study, or a rise in plasma noradrenaline (von Euler, Franksson & Hellstrøm, 1954). This presumably represents an increased release of noradrenaline from sympathetic nerve terminals in response to the drop in plasma catecholamine concentration resulting from adrenalectomy. Immunosympathectomy, which leaves adrenal medullary cells intact, but causes degeneration of noradrenergic nerve terminals, was found to cause only a moderate decrease in urinary noradrenaline metabolites (Caesar, Ruthven & Sandler 1969), while immunosympathectomy with adrenalectomy causes an increase in urinary NA (Carpi & Oliverio, 1964). These results can be explained by the findings of Berkowitz, Spector & Tarver, (1972), that immunosympathectomy causes almost complete depletion of the noradrenaline content of the heart, but has a smaller effect on vascular noradrenaline. Similar results were obtained with 6-hydroxydopamine (Berkowitz *et al.*, 1972). These experiments suggest that the sympathetic nerves to the blood vessels are a major source of urinary and therefore of plasma noradrenaline.

The rapid removal of noradrenaline from the blood stream was first demonstrated by Whitby, Axelrod & Weil-Malherbe (1961). Cession-Fossion (1974), using [^{3}H]-noradrenaline, but without distinguishing between noradrenaline and its metabolites, showed that radioactivity in the plasma was reduced to 7.4% 1 min after injection. In the present study plasma noradrenaline concentration was measured after the administration of an exogenous dose of 100 μg/kg body weight; taking rat plasma volume as 40 ml/kg (Huang & Bondurant, 1956), 4% of the administered dose is found in the plasma 1 min after injection whereas after DMI and NMN 43% of the original dose is still present after 1 min. Extrapolation to zero time raises these values to 6.2% and 48.8% respectively. This shows that the uptake processes blocked by DMI and NMN account for 42.6% of the removed noradrenaline. The rest may be accounted for by uptake and metabolism of noradrenaline by the lungs (Whitby *et al.*, 1961), liver and gut (Vane, 1969). Very little noradrenaline is removed by renal excretion, (Kopin & Gordon, 1963).

The experiments using 1 min swim-stress show that a brief activation of sympathetic nerves causes phasic changes in plasma noradrenaline concentration. Furthermore, it was found that the plasma noradrenaline concentration at the end of swim-stress varied with the temperature of the water.

The time course of noradrenaline entry into the circulation during a 1 min swim-stress is intermediate between a pulse intravenous injection of noradrenaline and the steady release from nerve terminals under normal conditions. The effect of DMI and NMN on plasma noradrenaline concentration varies in the three situations; it causes a 2.6 fold increase under resting conditions, a 6.6 fold increase during swim-stress and a 10 fold increase after exogenous noradrenaline. In addition to the differences in levels of noradrenaline in the plasma, the rate of decline also seems to vary with the method of its entry into the circulation. Thus the shortest half-life occurs after swim-stress with DMI and NMN while after the exogenous dose of noradrenaline it is longer, even in the absence of DMI and NMN. The plasma noradrenaline concentration after swim-stress alone (Figure 2c), suggests that noradrenaline release continues after the end of swim-stress and the rate of disappearance of noradrenaline could therefore not be calculated.

The present experiments indicate that in addition to neuronal and extraneuronal uptake which can be blocked by DMI and NMN, noradrenaline is also removed by processes that are unaffected by these drugs. The existence of these several removal processes accounts for the short half-life of noradrenaline in plasma. The results also show that changes in plasma noradrenaline reflect changes in the rate of release of transmitter and the method can therefore be applied to monitor transmitter release *in vivo*.

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