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0014-4754/89/080712-07$1.50 + 0.20/0

Extraadrenal adrenaline formation by two separate enzymes

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Received 2 January 1989; accepted 14 March 1989

Summary. Adrenaline (A) is synthesized in the adrenal medullae by the enzyme phenylethanolamine-N-methyltransferase (PNMT). After surgical removal of the adrenal medullae tissue A levels ranged from 22% of control in the heart to 125% of control in the liver. Use of a novel assay to measure tissue A formation revealed that many tissues can synthesize A using PNMT and another enzyme that N-methylates both noradrenaline and dopamine. These enzymes are non-neuronal, inducible and synthesize a major fraction of tissue and urine A.
Key words. Adrenaline; epinephrine; phenylethanolamine-N-methyltransferase; PNMT; epinine.

Adrenaline (A) (also called epinephrine) is synthesized in the adrenal glands by the enzyme phenylethanolamine-N-methyltransferase (PNMT). The enzyme PNMT is also found in very small amounts in the brain, in even smaller amounts in sympathetic ganglia and the heart. Almost all PNMT is found in the adrenals [1] but when rats have their adrenal medullae surgically removed, A is still present in their urine [2] and increases during stress [3] and with time [4]. Bilateral adrenalectomy did not change urinary A in man or the A response to stress [5]. Adrenalectomized rats still synthesized renal and urinary A [6].
Noradrenaline (NA) is the physiologic substrate for PNMT; it is N-methylated to form A. Assays for PNMT use phenylethanolamine, or a related lipophilic amine, as substrate for PNMT so that an easily isolated product is formed. We have devised an assay for A forming activity (AFA) that uses NA as substrate. This permits assay of AFA by enzymes other than PNMT and investigation into the sources of extraadrenal A synthesis.

Methods

To assay tissue A forming activity (AFA), 50 µl of supernatant from centrifuged tissue homogenate was incubated for 90 min at 25 °C in the presence of 2 M Tris with 5% EDTA, pH 8.6, 2.2 µCi ^{3}H-S-adenosylmethionine, and 1 mg NA or dopamine. Each sample was then shaken for 5 min with 100 mg Al_2O_3 (alumina). The alumina was washed 4 times with 2 ml cold 1-mM dithiothreitol solution. Catechols were then eluted from the alumina with 0.6 ml of 0.1 M perchloric acid and the acid supernatant was transferred to polystyrene tubes. Remaining ^{3}H-S-adenosylmethionine was precipitated with a solution of A, S-adenosylmethionine and phosphotungstic acid. The tubes were centrifuged at 8500 g and the supernatant was transferred to a scintillation vial. One mM dithiotheitol in 1 ml pH 7.5 phosphate buffer was then added followed by 200 µl of scintillant and 4 ml of 1% diethylhexyl phosphoric acid in toluene. Finally, vials were capped, shaken, and counting was done by liquid scintillation spectrometry.
The assay for AFA has a sensitivity (twice blank) of 14 fmol of A formed. The assay was linear from 0.014 pmol/h to 6 pmol/h ($r = 0.998$) using dilutions of rat adrenal medulla in 1 mg bovine serum albumin/ml. There was a within assay coefficient of variance of 2% and between assay coefficient of variance of 18% using 0.045 pmol/h of AFA. The addition of partially purified rat liver catechol-O-methyltransferase had no effect on the assay. AFA is expressed as pmol of A formed per gram of wet tissue per hour of incubation time at 25 °C. Catecholamines were measured by the catechol-O-methyltransferase based radioenzymatic technique of Ziegler et al. [7].

Results

In experiment number 1, male Sprague-Dawley rats weighing 200–250 g underwent denervation by superior cervical ganglionectomy or renal nerve ablation. Denervation depleted tissue NA by 93–99%, yet had little effect on AFA of most tissues (fig. 1). In experiment number 2, seven rats underwent bilateral adrenal demedullation and 6 rats had sham surgery. Twelve days later AFA of the cardiac ventricle was 31 ± 3 pmol A/gm/h in the adrenal medullectomized group and

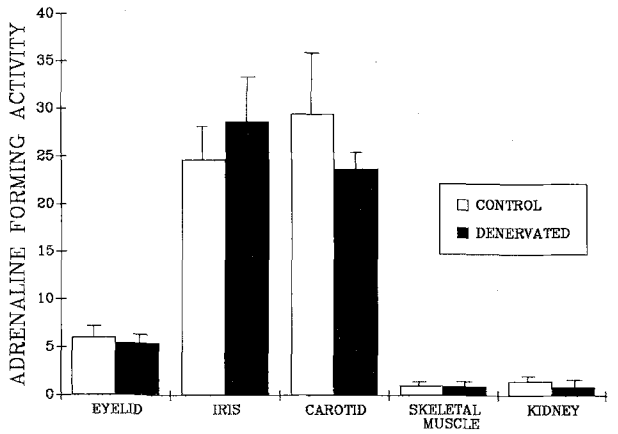

Figure 1. A forming activity of tissues while innervated and after denervation in experiment number 1. Activity is expressed as pmol of A formed from NA per gram of tissue per hour. There were 5 to 7 rats per group, but denervation failed to significantly alter A forming activity in any of the tissues studied.

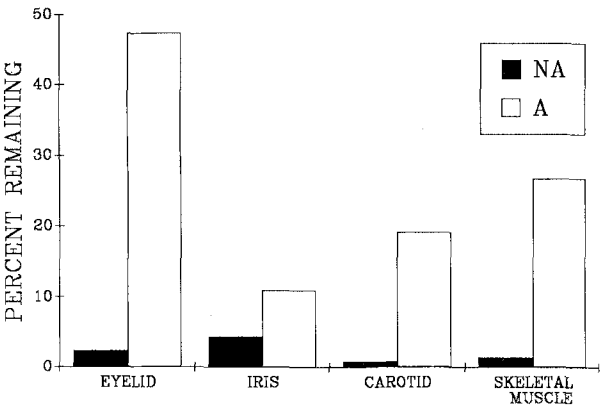

Figure 2. Percent of NA and A remaining in tissues after denervation, adrenalectomy, and reserpine administration in experiment number 3.

20 ± 3 in the sham operated group ($p < 0.05$). Liver AFA was similarly elevated in the medullectomized group (24.5 ± 1.3 vs 18.5 ± 1.1; $p < 0.01$).

PNMT is found in adrenal and nerves, but AFA of most organs was not removed by sympathetic nerve ablation. PNMT is inhibited by (10^{-4} M) SKF 29 661 and is much more active at N-methylating β-hydroxylated phenylethanolamines such as NA than non-β-hydroxylated phenylethylamines such as dopamine. AFA of the adrenal, brain stem and cardiac atrium had these characteristics of PNMT, while the AFA of cardiac ventricle and most other organs was poorly inhibited by SKF 29 661 and methylated both NA and dopamine (table).

In experiment number 3, rats were adrenalectomized and unilaterally superior cervical ganglionectomized. Three days later, and 24 h prior to sacrifice, rats were injected with reserpine 5 mg/kg i.p. to deplete neuronal catecholamine stores. This reduced the tissue NA of denervated tissues by about 97%, plasma A by 93% but tissue A by only 35–81% (fig. 2).

Rats in experiment number 1 underwent unilateral renal nerve ablation and adrenal demedullation 12 days prior to study. Kidney A levels were 0.3 ± 0.1 in control animals, 0.2 ± 0.006 in the innervated kidney of adrenal demedullated animals and 0.3 ± 0.1 ng/g in the denervated kidney of adrenal demedullated animals. In contrast, kidney NA fell by 95% in the denervated kidneys. Urine A was 6.8 ± 2 ng/ml in control animals and 7.3 ± 2 ng/ml from the denervated kidney of adrenal demedullated animals. In 7 rats there was a correlation between tissue and urine A levels from the denervated kidney ($r = 0.85$, $p < 0.05$). Furthermore, the amount of A present in 30 samples of denervated tissues from 5 rats correlated with AFA of the tissues ($r = 0.58$, $p < 0.001$).

Discussion

Several studies have suggested that physiologically important amounts of A can be formed outside the adrenal[2, 3, 5, 6]. Use of an assay that measures actual A formation reveals why it was difficult to find one source of extraadrenal A appearing in plasma and urine; small amounts of A can be synthesized by most tissues. This A is synthesized by at least 2 enzymes. One of these is PNMT, an enzyme specific for β-hydroxylated phenylethanolamines such as NA and inhibited by SKF 29 661. Another is a more ubiquitous N-methyltransferase (NMT) that methylates both NA and the non-β-hydroxylated phenylethylamine dopamine and is much less affected by SKF 29 661. PNMT is the predominant A forming enzyme in the cardiac atria (table). The car-

Adrenaline forming activity (AFA) and epinine forming activity in rat tissues

	Adrenal	Brain stem	Kidney	Denervated muscle	Atria	Ventricle	Rat RBC	Human RBC
AFA pmol/g/h	18 593 ± 1767	34.3 ± 12.5	0.18 ± 0.03	3.48 ± 0.44	59.5 ± 7.5	5.36 ± 0.39	4.54 ± 3.27	26.2 ± 12.8
Epinine FA pmol/g/h	223 ± 34	0.17 ± 0.03	0.13 ± 0.003	0.86 ± 0.22	1.40 ± 0.30	2.72 ± 0.14	5.05 ± 1.32	6.55 ± 1.8
Epinine formed as % of A formed	1.2	0.5	72	24.7	2.4	51	111	25
% Inhibition of AFA by 10^{-4} M SKF 29 661	90	77	18	24	67	13	5	47

AFA = adrenaline forming activity; Epinine FA = epinine forming activity; RBC = red blood cell. Data is from 4 to 7 animals in each group and is shown as mean ± SEM.

diac ventricles are less heavily innervated and their A forming activity is predominantly from NMT (table). Sympathetically denervated tissues retain most or all of their A forming activity (fig. 1) and this A forming activity has the characteristics of NMT. These tissues appear to continue to synthesize A after sympathectomy because they contain A even after adrenal demedullation (fig. 2) and the A levels correlate with NMT activity. NMT activity increased after A levels were lowered by adrenal demedullation. Induction of NMT by low A levels may explain why A levels of rats increase with time following removal of the adrenal medullae[4] and why no symptoms develop when the adrenal medullae are absent.

A stimulates β_2 adrenergic receptors much better than do NA or dopamine. β_2 receptors are present on sympathetic nerves (where they enhance NA release) and blood vessels (where they vasodilate). Local A formation could affect the innervation and blood supply of many organs. Other endocrine systems, such as the renin angiotensin system, have local paracrine functions. A can be synthesized by many tissues and can stimulate adrenergic receptors in the same tissues, so A could have paracrine functions as well as endocrine functions. We found A forming activity in both human red (table) and white blood cells, so NMT activity can also be studied in humans.

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0014-4754/89/080718-03$1.50 + 0.20/0

Depletion of hypothalamic norepinephrine reduces the fever induced by polyriboinosinic acid: polyribocytidylic acid (Poly I:Poly C) in rats[1]

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Received 17 January 1989; accepted 7 April 1989

Summary. Administration of either Poly I:Poly C (0.05–0.50 μg) or norepinephrine (2–8 μg) into the anterior hypothalamic area produced a dose-related fever in rats. The fever induced by Poly I:Poly C was attenuated after selective depletion of norepinephrine in the hypothalamus. However, selective depletion of hypothalamic norepinephrine did not affect the fever induced by intrahypothalamic norepinephrine. The data indicate that Poly I:Poly C may act to induce fever through the endogenous release of norepinephrine from the rat's hypothalamus.
Key words. Hypothalamus; norepinephrine; fever; pyrogen; polyriboinosinic acid; polyribocytidylic acid.

Evidence has accumulated to indicate that human alpha interferon (IFN) meets the criteria for being an endogenous pyrogen[2–4]. It produces a brisk, monophasic fever following injection, is free of endotoxin, and increases the production of prostaglandin E-2 from brain tissue in vitro and in vivo. Furthermore, when the homopolymer duplex of Poly I:Poly C, an IFN inducer, was injected i.v., it was shown to induce fever accompanied by the appearance of high titers of IFN in rabbits[5]. In contrast, equivalent doses of Poly I:Poly C alone were not pyrogenic. Recently, both in the rabbit[6] and the rat[7], microinjection of Poly I:Poly C or IFN into the anterior hypothalamic area was also shown to produce fever.

It should be noted that the latency of onset for fever was about 60 min after an intravenous dose, or about 30 min after an intrahypothalamic dose of Poly I:Poly C or IFN. Such a long latency indicates that IFN or its inducer may act through the endogenous release of intermediary pyrogenic factors to induce fever. Indeed, it has been shown that the IFN-induced or Poly I:Poly C-induced fever may be due to the local release of a prostaglandin or a protein factor of unknown chemical nature in the hypothalamic area. Further, a recent report demonstrated that depletion of norepinephrine in the rat's hypothalamus reduced the fever induced by prostaglandin E-2[8]. This raises the possibility that the noradrenergic pathways in the hypothalamus are involved in the genesis of fever induced by IFN or its inducer Poly I:Poly C. In order to deal with this question, in the present study 6-hydroxydopamine (6-OHDA) was used to destroy the