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Influence of ω-amino-acids on blood pressure, catecholamine stores and the pressor response to physostigmine in the rat

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The effects of aliphatic ω-amino-acids of various chain lengths, i.e. glycine, γ-aminobutyric acid, δ-amino-valeric acid, ε-aminocaproic acid and 7-amino-oenanthic acid, on blood pressure and myocardial catecholamine stores in the renal hypertensive rat, and the pressor response to physostigmine in the normotensive rat were studied. The effects of the ω-amino-acids were compared with those of guanethidine and α-methyldopa. ε-Aminocaproic acid, like guanethidine and α-methyldopa, produced a fall in blood pressure, a decrease in catecholamine stores and inhibited the pressor response to physostigmine. GABA reduced blood pressure, but did not affect the other parameters. δ-Amino-valeric acid reduced blood pressure and depleted catecholamine stores slightly, but did not inhibit the pressor response to physostigmine. The other ω-amino-acids were inactive.

EPSILON aminocaproic acid (EACA) is currently in clinical use as an antifibrinolytic agent. Depletion of myocardial noradrenaline stores in the rat and mouse after treatment with EACA has been described by Lippmann, Wishnick & Buyske (1965), and this effect has been related to inhibition of the dual amine uptake concentration mechanism of the adrenergic neurons (Obianwu, 1967a). In addition, EACA has been reported to produce adrenergic nerve blockade (Andén, Henning & Obianwu, 1968).

Another ω-amino-acid, γ-aminobutyric acid (GABA), has been reported to have an antihypertensive effect in man (Takahashi, Tiba & others, 1956), which is attributed to an influence on the central nervous system rather than to an effect on peripheral sympathetic transmission (Belloni, Savioli & Barbieri, 1966).

The present paper describes the effects of ω-amino-acids of various chain lengths on blood pressure and catecholamine stores in the renal hypertensive rat. In addition, the effect of these compounds on the pressor response to noradrenaline and physostigmine was studied in the normotensive rat. The effects are compared with those of two antihypertensive agents which are known to cause a depletion of noradrenaline from its catecholamine storage site, i.e. guanethidine and α-methyldopa.

Experimental

METHODS

Antihypertensive effects in renal hypertensive rats. Male rats were made hypertensive by constriction of the left renal artery. After stable near-systolic blood pressure levels of 170 mm Hg and above had been reached, the animals were divided into groups of 6–23 rats each with similar mean blood pressure levels. Blood pressure was measured at the beginning of the treatment period, 2 and 24 hr after the first administration, 24 hr

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after the second administration, 2 and 24 hr after the third administration and 2 hr after the fourth administration. The plethysmographic method of Byrom & Wilson (1938) was used.

The animals were treated daily for 4 days with the following compounds and doses: guanethidine, 3 mg/kg/day subcutaneously; DL-α-methyldopa (α-methyldopa), 300 mg/kg/day orally; glycine, 1000 mg/kg/day intraperitoneally; GABA, 300 mg/kg/day orally, 1000 mg/kg/day intraperitoneally; δ-aminovaleric acid (DAVA), 1000 mg/kg/day intraperitoneally; EACA, doses as for GABA; 7-amino-oenanthic acid (7-AOA), 1000 mg/kg/day intraperitoneally.

The effect of a single intraperitoneal dose of 1000 mg/kg EACA on blood pressure was followed for 14 hr after administration. In parallel groups of 13 hypertensive rats each, blood pressure was measured 2, 4, 6, 8 and 14 hr after administration of EACA or 0·9% saline.

Noradrenaline concentrations in heart and brain of renal hypertensive and normotensive rats. Twenty four hr after the fourth administration of α-methyldopa or 2–2½ hr after the fourth administration of the other compounds, the animals were bled and the organs removed. The noradrenaline of pooled tissue homogenates of two animals was extracted twice with 10% trichloroacetic acid, adsorbed onto alumina at pH 8·4, eluted with 0·25N HCl and measured fluorometrically (Euler & Orwén, 1955; Euler & Lishajko, 1959).

In a separate experiment, the effect of a single intraperitoneal dose of 1000 mg/kg EACA on myocardial catecholamine concentrations in normotensive rats was determined at various intervals until catecholamine levels returned to normal.

Pressor responses to noradrenaline and physostigmine. Normotensive male rats were treated daily for 4 days. Guanethidine (3 mg/kg/day) was injected subcutaneously and α-methyldopa (300 mg/kg/day) was given orally. Glycine, GABA, DAVA, EACA and 7-AOA (1000 mg/kg/day) were administered intraperitoneally. Two hr after the fourth administration the animals were anaesthetized with urethane (1·7 g/kg, intraperitoneally) and blood pressure in the carotoid artery was recorded with a mercury manometer. Noradrenaline (6 μg/kg) or physostigmine salicylate (150 μg/kg) were injected into a canulated jugular vein. The maximal increases in blood pressure produced by noradrenaline or by physostigmine in parallel groups of treated and untreated control animals were compared. For details of methods above, see Brunner, Hedwall, & others (1967). α-Methyldopa was administered as a suspension in acacia; all other substances were dissolved in 0·9% saline solution. The statistical methods of Lord (1947) and Hogben (1964) were used.

Results

Antihypertensive effects in renal hypertensive rats. The ω-amino-acids, GABA and EACA, produced no significant change in blood pressure after oral administration (Table 1). After intraperitoneal administration of 1000 mg/kg, GABA, DAVA and EACA reduced blood pressure to approximately the same extent as 300 mg/kg/day α-methyldopa given orally, while

TABLE 1. ANTIHYPERTENSIVE EFFECTS IN RENAL HYPERTENSIVE RATS

Treatment Initial blood pressure value mm Hg (n)	Change in blood pressure in mm Hg					
	2 hr after 1st admin.	24 hr after 1st admin.	24 hr after 2nd admin.	2 hr after 3rd admin.	24 hr after 3rd admin.	2 hr after 4th admin.
0·9% NaCl 2 ml/kg day oral 209±4 (14)	−5±5	−15±5	−15±5	−19±4	−19±5	−26±4
2 ml/kg day i.p. 213±3 (16)	−6±4	−14±3 (n=10)	−12±5 (n=10)	−10±3	−8±4	−21±5
Guanethidine 3 mg/kg/day s.c. 188±2 (12)	−34±6***	−38±4***	−48±4***	−70±4***	−52±4***	−69±4***
α-Methyldopa 300 mg/kg day oral 188±3 (23)	−20±3**	−25±6	−29±5*	−40±4***	−34±4***	−39±4*
Glycine 1000 mg/kg day i.p. 202±4 (6)	−22±3*	−11±4	−7±2	−23±2	−12±3	−24±6
GABA 300 mg/kg day oral 205±5 (7)	−16±6	+4±5	+6±5	−16±5	+13±5	−11±5
1000 mg/kg day i.p. 205±7 (7)	−54±6***	+6±4	0±3	−43±6***	−6±7	−42±8*
DAVA 1000 mg/kg day i.p. 213±4 (6)	−39±6***	0±16	−16±9	−53±5***	−12±8	−34±6
EACA 300 mg/kg/day oral 205±4 (7)	−6±3	−4±4	+5±5	−8±6	−7±6	−11±7
1000 mg/kg/day i.p. 206±3 (13)	−18±4	−8±3	−1±4	−36±5***	−2±6	−42±5*
7-AOA 1000 mg/kg/day i.p. 202±7 (6)	−8±3	−8±6	−8±5	−25±6	−15±2	−24±3

Values are means ± s.e.
n = number of animals.
* = significant at $P<0.05$, ** = significant at $P<0.01$, *** = significant at $P<0.001$ as compared to the control values.

7-AOA was inactive. Glycine produced a marginal but statistically significant fall in blood pressure only on the first day of treatment. The antihypertensive effects of GABA, DAVA and EACA, in contrast to those of α-methyldopa and guanethidine, were of relatively short duration. A significant decrease in blood pressure was seen 2 hr after the first administration of GABA and DAVA while 22 hr later blood pressure had returned to hypertensive levels. A similar fall in blood pressure was measured 2 hr after administration on the third and fourth day of treatment. The antihypertensive effect of EACA seemed to develop more slowly. Decreases in blood pressure were seen only 2 hr after administration on the third and fourth day of treatment (Table 1).

Noradrenaline concentrations in heart and brain of renal hypertensive rats. The noradrenaline concentrations in heart and brain of animals

treated intraperitoneally with the ω-amino-acids or with guanethidine (subcutaneously) or α-methyldopa (orally) were determined. The results are shown in Table 2. Glycine, GABA and 7-AOA did not influence myocardial catecholamine concentrations. EACA, on the other hand, produced a marked depletion which surpassed that produced by α-methyldopa or guanethidine. DAVA reduced myocardial catecholamine content slightly. A decrease in brain catecholamine concentration was produced only by α-methyldopa.

TABLE 2. NORADRENALINE CONCENTRATIONS IN HEART AND BRAIN OF RENAL HYPERTENSIVE RATS

	Noradrenaline μg/g wet weight			
4 days treatment with	n	Heart	n	Brain
0·9% NaCl	17	0·76 ± 0·031	17	0·410 ± 0·015
Guanethidine 3 mg/kg/day s.c.	6	0·14 ± 0·015***	6	0·366 ± 0·031
α-Methyldopa 300 mg/kg/day oral	4	0·16 ± 0·010***	4	0·114 ± 0·006***
Glycine 1000 mg/kg/day i.p.	2	0·79	3	0·407 ± 0·018
GABA 1000 mg/kg/day i.p.	3	0·68 ± 0·120	3	0·431 ± 0·023
DAVA 1000 mg/kg/day i.p.	6	0·48 ± 0·036**	6	0·382 ± 0·020
EACA 1000 mg/kg/day i.p.	3	0·099 ± 0·030***	3	0·431 ± 0·056
7-AOA 1000 mg/kg/day i.p.	3	0·55 ± 0·110	3	0·398 ± 0·017

Values are means ± s.e.
n = number of extracts.
** = $P<0.01$, *** = $P<0.001$ as compared to the control values.

Influence of a single dose of EACA on blood pressure in renal hypertensive rats and myocardial catecholamine stores in normotensive rats. As seen in the previous experiment, a single intraperitoneal dose of EACA had no influence on blood pressure (Table 3). A progressive decrease in blood pressure seen after a single injection of EACA was also found in saline-treated control animals, and is probably the result of repeated ether anaesthesia. Myocardial catecholamine levels, however, decreased

TABLE 3. EFFECT OF A SINGLE INTRAPERITONEAL INJECTION OF EACA ON BLOOD PRESSURE IN RENAL HYPERTENSIVE RATS

Treatment	n	Initial blood pressure mm Hg	Decrease in blood pressure (Δ mm Hg) after				
			2 hr	4 hr	6 hr	8 hr	14 hr
0·9% NaCl 2 ml/kg i.p.	13	233 ± 4	−20 ± 5	−34 ± 4	−41 ± 6	−40 ± 5	−21 ± 5
EACA 1000 mg/kg i.p.	13	210 ± 3	−27 ± 6	−39 ± 6	−36 ± 9	−42 ± 8	−18 ± 10

Values are means ± s.e.
n = number of animals.

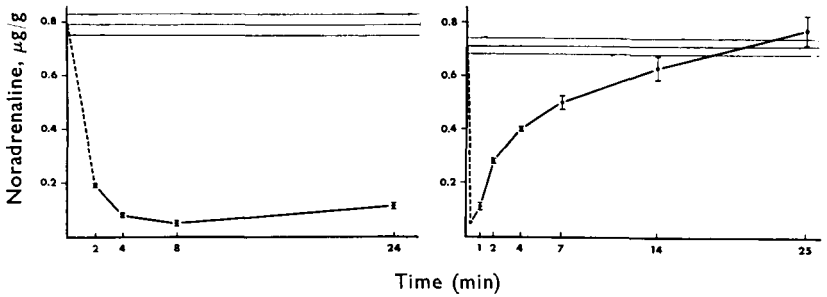

FIG. 1. Effect of a single intraperitoneal injection of 1000 mg/kg EACA on myocardial noradrenaline concentrations in normotensive rats. Points represent means of 3–5 extracts of two hearts each; vertical bars s.e. Control values are given as horizontal lines.

rapidly: the lowest concentrations were found 4–24 hr after administration. Within 4 days the catecholamine levels had been restored to about 50% of control values, and full recovery was found after 3 weeks (Fig. 1).

Pressor responses to noradrenaline and physostigmine. Potentiation of the noradrenaline effect and inhibition of the pressor response to physostigmine was produced by guanethidine, α-methyldopa and EACA. Glycine, GABA, DAVA and 7-AOA had no influence on the pressor responses (Table 4).

TABLE 4. PRESSOR RESPONSES TO NORADRENALINE AND PHYSOSTIGMINE IN NORMOTENSIVE RATS

4 days treatment with	n	Pressor response (Δ mm Hg) to Noradrenaline 6 μg/kg i.v.	Pressor response (Δ mm Hg) to Physostigmine salicylate 150 μg/kg i.v.
0·9% NaCl	12	58±4	37±3
Guanethidine 3 mg/kg/day s.c.	9	70±4*	22±5*
0·9% NaCl	18	58±3	45±4
α-Methyldopa 300 mg/kg/day oral	20	72±4***	32±3*
0·9% NaCl	10	40±4	52±5
Glycine 1000 mg/kg/day i.p.	5	34±8	61±12
GABA 1000 mg/kg/day i.p.	10	34±3	42±6
0·9% NaCl	7	45±3	32±7
DAVA 1000 mg/kg/day i.p.	10	54±4	25±4
0·9% NaCl	10	40±4	52±5
EACA 1000 mg/kg/day i.p.	9	57±5*	35±2***
7-AOA 1000 mg/kg/day i.p.	5	36±4	51±5

Values are means ± s.e.
n = number of animals.
* = significant at $P<0.05$, ** = significant at $P<0.01$, *** = significant at $P<0.001$.

Discussion

Obianwu (1967a, b) showed that EACA, like guanethidine, inhibits the dual amine uptake-concentration mechanisms of the adrenergic neurons. He also pointed out that the adrenergic nerve blockade induced by EACA differs from that produced by guanethidine. The short-lasting antihypertensive effect of EACA would tend to support this suggestion. EACA is absorbed and eliminated rapidly in man (Dupont, 1965). Assuming that this is also true in the rat, variations in the intensity of the antihypertensive effect may reflect variations in blood EACA concentrations.

Myocardial catecholamine levels, however, do not vary to such degree. A single intraperitoneal injection of EACA reduced myocardial catecholamine stores in normotensive rats for at least 24 hr to approximately the same extent as 4 days treatment of hypertensive rats. The catecholamine depletion found 4 and 8 hr after 1000 mg/kg intraperitoneally was slightly more pronounced than that which Lippmann & others (1965) have described after oral administration of 500 mg/kg. It is more marked than that produced by guanethidine or α-methyldopa. In contrast, blood pressure was not significantly influenced by a single intraperitoneal injection of EACA. Subsequent to an initial sympathomimetic phase, EACA produced a 20% decrease in blood pressure 6 hr after administration of 2 g/kg i.p. in the normotensive, non-anaesthetized rat (Andén, Henning & Obianwu, 1968). In our experiments neither an initial increase in blood pressure nor a later decrease in blood pressure was observed. These differences may be due to the differing EACA doses used or to the influence of the light ether anaesthesia, which decreased blood pressure in saline-treated control rats.

DAVA reduced the concentration of myocardial catecholamines by about 30% after 4 days treatment. None of the other ω-amino-acids studied influenced myocardial catecholamine contents significantly. Likewise, it has been shown that β-alanine does not reduce myocardial catecholamine concentrations (Lippmann & others, 1965).

Inhibition of adrenergic transmission as estimated by reduction of the pressor response to physostigmine, was seen only after EACA, α-methyldopa and guanethidine. According to the results of Varagić (1955), the pressor response to physostigmine in the urethane-anaesthetized rat may be attributed to central sympathetic stimulation. A reduction in this pressor response has been related to inhibition of central sympathetic centres or impairment of peripheral sympathetic transmission (Lešić & Varagić, 1961). The potentiation of the pressor response to noradrenaline by EACA indicates that inhibition of adrenergic transmission is not the result of adrenergic α-receptor blockade.

GABA and DAVA, which had an effect on blood pressure similar to that of EACA, had little or no influence on myocardial catecholamine stores and did not affect the pressor response to physostigmine. Thus, GABA and DAVA seem to have a different mechanism of antihypertensive action. The other ω-amino-acids, glycine and 7-AOA, did not influence any of these parameters. The slight effect of glycine on blood

pressure on the first day of treatment is probably without pharmacological significance.

A single administration of EACA produced rapid and long-lasting depletion of myocardial noradrenaline stores. Likewise, Obianwu (1967a) reported an impairment of membrane and storage particle uptake mechanisms within a few hours after giving a single intraperitoneal dose of 1000 mg/kg EACA. The antihypertensive effect of EACA on the other hand, developed only after repeated injections and does not seem to be directly correlated with the depletion of peripheral catecholamine stores or with impairment of adrenergic neuron uptake-storage mechanisms.

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