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The effect of ascorbic acid on the cerebral and adrenal catecholamine content in the male rat

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The intraperitoneal administration of ascorbic acid (500 mg/kg) to rats produces a significant decrease in the concentration of dopamine, and an increase in noradrenaline, in the cerebral hemispheres, cerebellum, diencephalon, mesencephalon and medulla oblongata. The only change in the concentration of adrenaline was a decrease observed in the cerebral hemispheres. Ascorbic acid produces an increase in the concentration of ascorbic acid in the brain; this rise was not observed in the adrenal medulla where the concentrations of catecholamines were also unchanged.

ASCORBIC acid increases significantly the concentration of catecholamines, expressed as noradrenaline, in cerebral hemispheres, cerebellum and mesencephalon of rats (Izquierdo, Jofré & Dezza, 1964). We have since found that the acid decreases significantly the adrenaline concentration but does not modify the noradrenaline contents in the guinea-pig heart (Izquierdo & Jofré, 1965).

According to McLean & Cohen (1963) ascorbic acid releases adrenaline from adrenal granules *in vitro*. Levin, Levenberg & Kaufman (1960) and Levin & Kaufman (1961) considered that the acid participated in the β-hydroxylation of dopamine in the adrenal gland.

These results, together with the discrepancy between our findings on guinea-pig heart (Izquierdo & Jofré, 1965) and on rat brain (Izquierdo & others, 1964), led us to reinvestigate the effect of ascorbic acid on the cerebral and adrenal catecholamine contents in the rat.

Experimental

One hundred and thirty adult male albino rats, weighing 84–126 g, were used. They were killed by decapitation and the dopamine, noradrenaline, adrenaline and ascorbic acid contents or the amines or acid alone in brain structures and in the whole adrenal glands were estimated. There were 41 normal rats; there were 4 groups, each of 16 animals, killed 5, 10, 20 and 60 min after intraperitoneal injection of ascorbic acid (500 mg/kg). A further 4 groups, this time of 2 rats each, were injected intraperitoneally with nialamide (100 mg/kg) 4 hr before death, and pyrogallol (10 mg/kg) and ascorbic acid in separate injections 20 min before death, in which the amines only were assessed. Finally there were 4 groups of 2 rats each, and 3 groups of 3 rats each, killed 20 min after injection of ascorbic acid in which the acid alone was assessed.

Drugs used were: ascorbic acid (Roche) dissolved in twice distilled water and adjusted to pH 5·5–6·0 with 5N sodium hydroxide; nialamide (Pfizer); pyrogallol (Mallinckrodt). Solutions were prepared extemporaneously for each animal.

Brains were removed, washed immediately with cold Tyrode solution

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and dissected into component parts on ice. Adrenals and the adrenal-medulla were similarly treated. The structures were weighed and then adrenaline and noradrenaline were determined spectrofluorimetrically according to Bertler, Carlsson & Rosengren (1958), dopamine according to Laverty & Sharman (1965), and ascorbic acid by the photocolorimetric method of Roe (1954).

Results

From Table 1 it can be seen that ascorbic acid decreased dopamine and at the same time increased noradrenaline in some cerebral structures, as early as 5 min after injection. The effect increased at 10 min and reached a maximum at 20 min in all the brain structures. At 60 min there was still a decreased content of dopamine in hemispheres, mesencephalon, pons and medulla oblongata, but with no corresponding increase

TABLE 1. EFFECT OF THE INTRAPERITONEAL ADMINISTRATION OF 500 MG/KG OF ASCORBIC ACID ON THE CONTENT OF THE CATECHOLAMINES IN DIFFERENT PARTS OF THE BRAIN AND ADRENAL GLANDS OF THE RAT

		DA	NA	A	DA	NA	A	DA	NA	A
	Wt (g)	Hemispheres			Cerebellum			Diencephalon		
Normal	103 (24)* (11)†	0·21 ±0·03	0·16 ±0·03	0·04 ±0·002	0·18 ±0·04	0·12 ±0·01	0·05 ±0·01	1·66 ±0·12	0·34 ±0·02	0·11 ±0·03
20 min	113 (16) (4)	0·06 ±0·01 P≪0·001 73·2 (D)	0·20 ±0·01 P<0·01 22·9 (I)	0·01 ±0·001 P≪0·001 83·2 (D)	0·06 ±0·0054 P≪0·001 69·3 (D)	0·17 ±0·01 P<0·01 41·9 (I)	0·07 ±0·01 P<0·05 54·9 (I)	0·89 ±0·07 P≪0·001 46·4 (D)	0·51 ±0·005 P≪0·001 48·7 (I)	0·14 ±0·01 P<0·01 25·5 (I)
60 min	104 (16) (4)	0·15 ±0·01 P<0·02 26·4 (D)	0·16 ±0·01 4·4	0·04 ±0·004 7·3	0·14 ±0·01 22·7	0·13 ±0·01 6·5	0·05 ±0·001 P<0·01 13·7 (I)	1·44 ±0·13 13·1	0·35 ±0·019 2·8	0·12 ±0·004 6·0
		Mesencephalon			Pons-medulla oblongata			Adrenal glands		
Normal	103 (24)(11)	0·80 ±0·07	0·31 ±0·03	0·05 ±0·01	0·72 ±0·06	0·42 ±0·04	0·09 ±0·003	0·03 ±0·004	0·13 ±0·02	0·52 ±0·05
20 min	113 (16) (4)	0·57 ±0·03 P≪0·001	0·49 ±0·04 P<0·02	0·07 ±0·006	0·45 ±0·03 P≪0·001	0·69 ±0·02 P≪0·001	0·11 ±0·01	0·03 ±0·003	0·12 ±0·008	0·55 ±0·02
		29·7 (D)	57·4 (I)	25·2 (I)	37·7(D)	58·5 (I)	16·4 (I)	13·4 (I)	7·5 (D)	4·5 (I)
60 min	104 (16) (4)	0·69 ±0·02 P<0·01 13·4 (D)	0·35 ±0·03 12·6 (I)	0·05 ±0·003 8·8 (D)	0·63 ±0·01 P<0·01 12·4 (D)	0·53 ±0·03 21·7 (I)	0·10 ±0·003 5·5 (I)	0·03 ±0·002 14·0 (I)	0·13 ±0·007 2·5 (D)	0·56 ±0·02 7·8 (I)

Values are means and standard errors in μg/g of fresh tissue, in adrenals μg/mg.
* Number of animals
† Number of determinations
(I) % increases
(D) % decreases
DA Dopamine
NA Noradrenaline
A Adrenaline

in noradrenaline, although adrenaline was still significantly high in the cerebellum.

Adrenaline content was decreased in the hemispheres after 5 min by injection of the acid but it was not modified in the mesencephalon, pons and medulla oblongata, and was increased significantly in the diencephalon and cerebellum.

On a percent basis, only in the diencephalon, and at 10 min after injection of acid, was dopamine decreased by 44%; this corresponds to the increase of noradrenaline (29%) and of adrenaline (16%) (Table 2).

TABLE 2. EFFECT OF THE ADMINISTRATION OF ASCORBIC ACID (500 MG/KG I.P.) ON THE CONTENT OF CATECHOLAMINES IN THE DIENCEPHALON

	Dopamine	Noradrenaline	Adrenaline
Normal	1·66 ±0·12*	0·34 ±0·02	0·11 ±0·03
At 10 min	0·92 ±0·01 44·3 (D) P<0·001	0·44 ±0·02 28·9 (I) P<0·02	0·13 ±0·01 15·7 (I)

* Values are means and standard errors of 4 determinations in μg/g of fresh tissue. (I) % increases; (D) % decreases.

After 20 min the noradrenaline increases were proportionally larger than the decreases in dopamine in the mesencephalon, pons and medulla oblongata.

The adrenal content of the three amines did not change significantly for 60 min after injection of the acid (Table 1).

Injection of ascorbic acid produced an increase in its concentration in brain structures; and even more so in the whole adrenal glands (Table 3).

TABLE 3. ASCORBIC ACID CONTENT IN BRAIN AND ADRENAL GLANDS. NORMAL AND INJECTED WITH ASCORBIC ACID 500 MG/KG I.P.

	Normal	20 min	% increase
Brain hemispheres	82·40 ± 3·65*	125·35 ± 2·40	52†
Cerebellum	80·59 ± 4·00	145·85 ± 3·46	81†
Diencephalon	66·38 ± 2·95	105·25 ± 6·39	58†
Pons and medulla oblongata ..	41·32 ± 2·70	85·92 ± 3·26	108†
Mesencephalon	60·52 ± 2·57	102·95 ± 7·10	70†
Adrenal glands	471·78 ± 13·00	973·60 ± 6·58	106†

* Values are means and standard errors of 4 determinations in μg/g of fresh tissue.
† P<0·001.

TABLE 4. ASCORBIC ACID AND DOPAMINE CONCENTRATION OF ADRENAL GLANDS AND ADRENAL MEDULLA IN NORMAL RATS AND RATS INJECTED WITH ASCORBIC ACID 500 MG/KG I.P.

	Normal	20 min
	Ascorbic acid	
Adrenal gland	471·10 ± 12·57*	988·14 ± 14·10 109 (I) P<0·001
Adrenal medulla ..	368·66 ± 12·05	402·15 ± 5·32 9·1 (I)
Cortex (deduced value) ..	102·44	585·99 472 (I)
	Dopamine	
Adrenal gland	0·03 ± 0·004	0·03 ± 0·003 13·4 (I)
Adrenal medulla ..	0·03 ± 0·001	0·04 ± 0·003 6·8

* Values are means and standard errors of 4 determinations in μg/g of fresh tissue.
(I) % increases.

This increase reached its peak at 20 min and declined at 60 min. No modification of adrenal medullary ascorbic acid was detected (Table 4), thus the increase in adrenal ascorbic acid appeared to occur only in the cortex of the gland.

Twenty min after rats pretreated with nialamide and pyrogallol were given ascorbic acid, a significant increase in the concentration of noradrenaline and adrenaline occurred in the diencephalon alone compared with the concentration of the amines in animals not treated with nialamide or pyrogallol. This pretreatment did not modify the effect of the acid on the concentration of dopamine in the diencephalon or in the adrenals (Table 5).

TABLE 5. EFFECT OF THE ASCORBIC ACID (500 MG/KG I.P.) ADMINISTRATION ON THE DIENCEPHALON AND ADRENALS CONTENT OF CATECHOLAMINES IN RATS PREVIOUSLY TREATED WITH NIALAMIDE AND PYROGALLOL

	Diencephalon			Adrenal—gland		
	DA	NA	A	DA	NA	A
Normal	1·66 ±0·12	0·34 ±0·02	0·11 ±0·03	0·03 ±0·004	0·13 ±0·02	0·52 ±0·05
Ascorbic acid (20 min)	0·89 ±0·07 46·4 (D) P≪0·001	0·51 ±0·01 43·7 (I) P≪0·001	0·14 ±0·01 25·5 (I) P<0·02	0·03 ±0·003 11·3 (I)	0·12 ±0·01 7·5 (D)	0·55 ±0·02 4·6 (I)
Nialamide Pyrogallol Ascorbic acid (20 min)	0·92 ±0·05 44·8 (D) P<0·001	0·65 ±0·03 89·8 (I) P<0·001	0·17 ±0·01 49·8 (I) P<0·001	0·03 ±0·003 11·5 (I)	0·11 ±0·01 15·3 (D)	0·57 ±0·02 9·1 (I)

Values are means and standard errors of 4 determinations in μg/g of fresh tissue, in adrenal μg/mg.
(I) % increases.
(D) % decreases.

Discussion

The simplest explanation for the decrease in dopamine and the increase in noradrenaline produced by ascorbic acid may be the possible participation of the acid in the β-hydroxylating mechanism of dopamine (Levin & others, 1960; Levin & Kaufman, 1961). According to these authors, ascorbic acid acts as coenzyme to dopamine β-hydroxylase in adrenal gland *in vitro* (Levin & others, 1960; Kaufman, 1966). But, except in the diencephalon at 10 min after injection of the acid, close agreement between dopamine decrease and noradrenaline increase is not apparent. Furthermore, adrenaline also appears to be decreased in brain hemispheres (Table 1) and we found the acid not to affect the three amines in the adrenal gland. This suggests that, *in vivo*, factors may be involved other than those which participate in the *in vitro* effect of the acid on catecholamine concentration.

It has been suggested that the hypophyso-corticoadrenal system regulates the adrenal medullary synthesis of adrenaline (Wurtman & Axelrod, 1965; Axelrod, 1966). There is a relation between adrenal cortical function and response to catecholamines (Brodie, Davies & others, 1966). However, neither ACTH nor corticoids modify the adrenaline content of adrenal glands (Linét & Hertting, 1966). Our results

suggest the acid to have an effect independent of cortical and medullary function, inasmuch as the acid is markedly increased in cortical tissue after intraperitoneal injection, but there is no change in medullary ascorbic acid or in adrenal dopamine, noradrenaline or adrenaline. This is suggested by the fact that in brain structures, which are normally relatively low in ascorbic acid, its injection not only increases its concentration but also markedly affects catecholamine concentration.

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