Effect of disulfiram and ascorbic acid on catecholamine content in rat brain

Ascorbic acid in doses of 500 mg/kg (i.p.), provoked after 20 min a decrease of dopamine content and an increase of noradrenaline in different cerebral structures in the rat. A parallel significant increase of ascorbic acid occurs in these structures (Izquierdo, Jofré & Acevedo, 1968).

The enzyme dopamine-β-hydroxylase (DBH) requires oxygen and ascorbate as cofactors for the conversion of dopamine to noradrenaline (Levin & Kaufman, 1961).

Levin, Levenberg & Kaufman (1960) and Goldstein, Anagnoste & others (1964) agree that ascorbic acid, besides being a necessary cofactor for DBH activity, favours the *in vitro* reduction of disulfiram, an inhibitor of DBH activity, to diethyldithiocarbamate (DDC), which participates in the inhibitory process of the enzyme in rat brain (Carlsson, Lindqvist & others, 1966).

DBH activity is inhibited either by disulfiram, because DDC chelates with the copper in the enzyme during 24 h (Musacchio, Goldstein & others, 1966), or by –SH inhibition (Lippman & Lloyd, 1971). We have examined the interaction between disulfiram and ascorbic acid 24, 48, 72 h after injection of the inhibitor, and measured the dopamine and noradrenaline content of cortex, cerebellum, diencephalon, mesencephalon and pons-medulla oblongata.

106 male albino rats (150 $\pm$ 30 g) of our colony were maintained in a 12 h light/dark cycle and were given disulfiram (Ayerst) (400 mg/kg, i.p.) in 3% arabic gum, 24, 48 and 72 h before, and ascorbic acid (Roche) (500 mg/kg, i.p., pH 5·5–6·0) 20 min before decapitation.

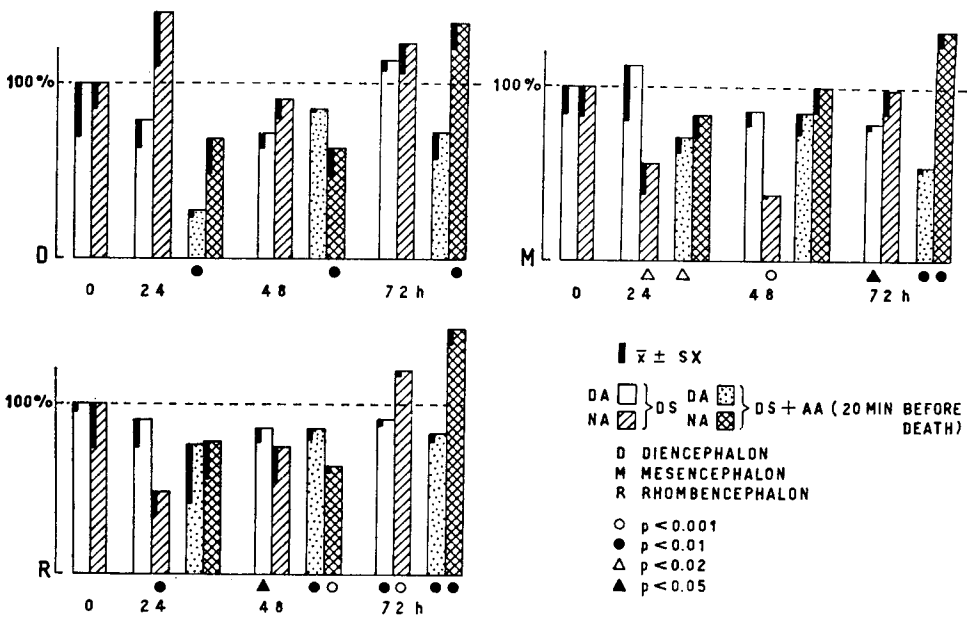

FIG. 1. Effect of disulfiram (DS) (400 mg/kg, i.p.) and ascorbic acid (AA) (500 mg/kg, i.p.) on catecholamine (NA) contents in rat brain (diencephalon, mesencephalon and pons-medulla oblongata). In cortex and cerebellum dopamine decreases ($P<0·01$) and noradrenaline increases ($P<0·05$).

Table 1. *Effect of ascorbic acid* (500 *mg/kg, i.p.*) *on noradrenaline content in mouse brain.*

	Control	Treated
Diencephalon	0·42 ± 0·05*	0·72 ± 0·03 ($P<0·001$)
Mesencephalon	0·46 ± 0·02	0·68 ± 0·01 ($P<0·01$)
Pons-Medulla oblongata ..	0·43 ± 0·05	0·57 ± 0·03 (N.S.)

* Values are means ± standard errors in μg/g of fresh tissue. Each determination made on a pool of 3 mice (n: 15).

Seven groups of 4–6 animals were used. On each occasion, one group was given inhibitor, another the inhibitor followed by ascorbic acid. There was also a control group. The results are in Fig. 1.

Dopamine was estimated according to Laverty & Sharman (1965) and noradrenaline was extracted according to von Euler & Lishajko (1961) and estimated (Bertler, Carlsson & others, 1958).

Disulfiram increased dopamine and decreased noradrenaline content after 2 h, the effect being maximal after 4 h. These responses were increased by ascorbic acid, though not significantly in the cerebral structures studied. The amount of brain noradrenaline remained low for more than 24 h, and reached normal after 72 h. Only in animals given disulfiram 72 h before death did ascorbic acid produce a significant increase of noradrenaline in all the cerebral structures. Furthermore, a similar effect on dopamine and noradrenaline contents was observed when ascorbic acid (500 mg/kg) was given to male Rockland mice (weight ±26 g).

The findings (Table 1) are not consistent with those reported by Hammarström (1966) and Sjöstrand (1970, and personal communication).

The discrepancies might be due to the higher doses of ascorbic acid in our experiments or to the strain of mice.

These findings show two main points: the first is that the inhibitory effect of disulfiram on DBH in rat brain persists for 72 h, the second is that ascorbic acid *per se*, as cofactor, participates either in activating the hydroxylating enzyme, or counteracting an endogenous inhibitor of DBH (such as that reported by Austin, Livett & Chubb (1967) and Nagatsu, Hidaka & Kuzuya (1967).

This work was supported by a Grant No. 3214/68 of Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina.

Cátedra de Farmacología Experimental,
Facultad de Farmacia y Bioquímica,
Junín 956, Buenos Aires,
Argentina.

J. A. IZQUIERDO
ISABEL J. JOFRÉ
CRISTINA ACEVEDO

September 6, 1971

REFERENCES

AUSTIN, L. B., LIVETT, G. & CHUBB, I. (1967). *Circ. Res., Suppl.* III, 111–117.
BERTLER, A., CARLSSON, A. & ROSENGREN (1958). *Acta physiol. scand.*, **44**, 273–292.
CARLSSON, A., LINDQVIST, M., FUXE, K. & HÖKFELT, T. (1966). *J. Pharm. Pharmac.*, **18**, 60–62.
EULER, U. S. VON & LISHAJKO, F. (1961). *Acta physiol. scand.*, **51**, 348–356.
GOLDSTEIN, M., ANAGNOSTE, B., LAUBER, E. & MCKEREGHAN, R. (1964). *Life Sci.*, **3**, 763–767.
HAMMARSTRÖM, L. (1960). *Acta physiol. scand.*, **70**, *suppl.* 289.
IZQUIERDO, J. A., JOFRÉ, I. J. & ACEVEDO, C. (1968). *J. Pharm. Pharmac.*, **20**, 210–214.
LAVERTY, R. & SHARMAN, D. F. (1965). *Br. J. Pharmac. Chemother*, **24**, 759–772.
LEVIN, E. Y. & KAUFMAN, S. (1961). *J. biol. Chem.*, **236**, 2043–2049.
LEVIN, E. Y., LEVENBERG, B. & KAUFMAN, S. (1960). *Ibid.*, **235**, 2080–2086.

LIPPMANN, W. & LLOYD, K. (1971). *Archs Int. Pharmacodyn. Thér.*, **189**, 348–357.
MUSACCHIO, J., GOLDSTEIN, M., ANAGNOSTE, B., POCH, G. & KOPIN, I. J. (1966). *J. Pharmac. exp. Ther.*, **152**, 56–61.
NAGATSU, T., HIDAKA, H. & KUZUYA, H. (1967). *Biochim. biophys. Acta*, **139**, 319–327.
SJÖSTRAND, S. E. (1970). *Acta physiol. scand., Suppl.* 356.

Experimental gastric ulcers and stomach tissue pepsin activity in the rat

Recently we demonstrated a relation between uropepsinogen excretion and experimental gastric ulcers in the rat (Coppi, Bonardi & Gaetani, 1971). We now report the pepsin activity of the stomach tissue of normal rats, of rats with reserpine or phenylbutazone-induced ulcers and in ulcerated rats also treated with atropine or oxyphencyclimine.

Male or female Sprague-Dawley rats, 160–170 g, had gastric ulcers produced by reserpine or phenylbutazone (Coppi & others, 1971). Groups of normal rats or with reserpine- or phenylbutazone-induced ulcers were treated orally with atropine sulphate or oxyphencyclimine (hydrochloride) both suspended in 5% acacia gum. The stomachs were removed 18 h after the reserpine or the last phenylbutazone dose, washed in running water, dried between filter paper, weighed, homogenized (1%) in 0·06N HCl by a blender (Sorvall Omni-Mixer) and used for pepsin assay (Anson, 1963). A portion of this homogenate was centrifuged at 3000 rev/min for 20 min

Table 1. *Relation between gastric ulcers and stomach tissue pepsin activity in the rat.* The pepsin activity and the morphological examination was made on rats 18 h after the reserpine or the second phenylbutazone dose. Atropine and oxyphencyclimine were given simultaneously with the reserpine or the second phenylbutazone dose.

Treatment	Pepsin activity (mg tyrosine/g N) mean ± s.e.		Morphological examination	
	1% Homogenate	Supernatant	Ulceration index mean ± s.e.	% Ulcerated animals
5% Acacia gum	13·6 ± 0·8 (20)	27·5 ± 1·7	0 (40)	0
Reserpine (5 mg/kg, i.p.)	5·9 ± 0·5 (20)†	9·0 ± 0·8†	2·0 ± 0·2 (39)†	93
Reserpine (5 mg/kg, i.p.) + atropine (10 mg/kg, orally)	5·6 ± 0·7 (19)†	10·7 ± 1·1†	1·5 ± 0·2 (40)†	75
Reserpine (5 mg/kg, i.p.) + oxyphencyclimine (10 mg/kg, orally)	9·7 ± 1·0 (20)†*	18·4 ± 2·2†*	0·5 ± 0·1 (40)†*	38
5% Acacia gum	17·4 ± 0·9 (27)	34·0 ± 1·9	0·2 ± 0·1 (30)	17
Phenylbutazone (100 mg/kg, orally twice in 8 h)	13·0 ± 1·0 (26)†	26·1 ± 2·1†	3·1 ± 0·2 (28)†	100
Phenylbutazone (100 mg/kg, orally twice in 8 h) + atropine (10 mg/kg orally)	17·3 ± 1·3 (26)*	36·4 ± 2·8*	1·8 ± 0·3 (29)†*	77
Phenylbutazone (100 mg/kg, orally twice in 8 h) + oxyphencyclimine (10 mg/kg, orally)	21·3 ± 1·1 (27)†*	43·6 ± 2·4†*	1·5 ± 0·3 (30)†*	60
5% Acacia gum	15·8 ± 1·3 (15)	31·7 ± 2·7	0·5 ± 0·2 (31)	36
Atropine (10 mg/kg, orally)	15·4 ± 1·5 (15)	29·1 ± 2·8	0·3 ± 0·1 (30)	23
Oxyphencyclimine (10 mg/kg, orally)	21·7 ± 1·2 (19)†	44·0 ± 3·0†	0 (30)†	0

* Significance relative corresponding control group; † significance relative to reserpine or phenylbutazone-treated group: *t*-test, ($P \leqslant 0{\cdot}01$).

Numbers of animals are in parentheses.