Department of Veterinary Pharmacology,
University of Glasgow,
Glasgow, U.K.

JAMES BOGAN

May 22, 1970

REFERENCES

BRODIE, B. B., MARK, L. C., PAPPER, E. M., LIEF, P. A., BERNSTEIN, E. & ROVENSTINE, E. A (1950). *J. Pharmac. exp. Ther.*, **98**, 85–96.

BUSH, M. T., MAZEL, P. & CHAMBERS, J. (1961). *Ibid.*, **134**, 110–116.

FOUTS, J. R. & BRODIE, B. B. (1956). *J. Pharmac. exp. Ther.*, **116**, 480–485.

MARK, L. C. & BRAND, L. (1963). *Bull. N.Y. Acad. Med.*, **40**, 476–482.

MARK, L. C., BRAND, L., KAMVYASI, S., BRITTON, R. C., PEREL, J. M., LANDRAU, M. A. & DAYTON P. C. (1965). *Nature, Lond.*, **206**, 1117–1119.

MEGIRIAN, R. (1964). *J. Pharmac. exp. Ther*, **144**, 331–336.

PRICE, H. L. KEVNAT, P. J., SAFER, J. N., CONNER. E. H. & PRICE, M. L. (1959). *Clin. Pharmac. Ther.*, **1**, 16–22.

SAIDMAN, L. J. & EGER, E. I. (1966). *Anesthesiology*, **27**, 118–126.

SHIDEMAN, F. E., KELLY, A. R. & ADAMS, B. J. (1947). *J. Pharmac. exp. Ther.*, **91**, 331–339.

SPECTOR, E. & SHIDEMAN, F. E. (1959). *Biochem. Pharmac.*, **2**, 182–196.

WINTERS, W. D., SPECTOR, E., WALLACH, D. P. & SHIDEMAN, F. E. (1955). *J. Pharmac. exp. Ther.*, **114**, 343–357.

2-Mercaptobenzothiazole, an inhibitor of dopamine β-hydroxylase

The presence of copper in purified preparations of dopamine β-hydroxylase and the functional role of cupric ions in the oxidative conversion of dopamine to noradrenaline have been reported (Friedman & Kaufman, 1965). The critical role of cupric ions in the activity of dopamine β-hydroxylase renders this enzyme vulnerable to inhibition by copper chelating agents. Chelation of the cupric ion is the probable mechanism for the inhibition of this enzyme by inhibitors which include disulfiram (Goldstein, Anagnoste & others, 1964), phenylethyldithiocarbamate (Jonsson, Grobecker & Gunne, 1967), tropolone (Goldstein, Lauber & McKereghan, 1964) and various aromatic and alkyl thioureas, including U-14,624 [1-phenyl-3-(2-thiazolyl)-2-thiourea] (Johnson, Boukma & Kim, 1969, 1970). The irreversible inhibition of a banana polyphenoloxidase, also a copper enzyme, by 2-mercaptobenzothiazole (MBT) (Palmer & Roberts, 1967) prompted our investigation of this drug as a potential inhibitor of dopamine β-hydroxylase.

In vitro inhibition of dopamine β-hydroxylase isolated from bovine adrenal medulla (Friedman & Kaufman, 1965) was measured (Goldstein, Prochoroff & Sirlin, 1965). The animals were CF-1 male mice, 18–22 g, and Upjohn Sprague-Dawley male rats, 180–190 g. The drugs were dissolved or suspended in 0·25% aqueous methylcellulose before intraperitoneal administration. Noradrenaline and dopamine in paired mouse brains were measured (Veldkamp, Johnson & Keasling, 1968). The repletion of rat myocardial noradrenaline from exogenous dopamine after the depletion of noradrenaline with metaraminol was examined as described by Nikodijevic, Creveling, & Udenfriend (1963). Myocardial noradrenaline was adsorbed onto alumina (Anton & Sayre, 1962), eluted with 0·5M acetic acid and assayed (von Euler & Floding, 1958).

Spontaneous motor activity was recorded in actophotometer cages (Woodward Research Corp.). Mice received MBT (300 mg/kg, i.p.) or vehicle and two mice from the same treatment group were placed in each cage. After an initial 10 min acclimation period, activity was recorded in each 30 min interval for 4 h.

MBT inhibited non-competitively dopamine β-hydroxylase *in vitro* 72% at 10^{-5}M and 47% at 5×10^{-6}M. In the same assay disulfiram produced 45% inhibition at 2×10^{-7} M and benzyloxyamine 30% at 5×10^{-4} M.

MBT (300 mg/kg, i.p.), lowered noradrenaline to approximately 60% of control after 1 and 2 h (Table 1). Dopamine levels were raised 24% above control concentrations at 2 h. Both noradrenaline and dopamine returned to control values at

Table 1. *Mouse brain catecholamine levels* 1, 2, *and* 4 *h after* 2-*mercaptobenzothiazole* (MBT), 300 mg/kg, i.p. All values are expressed as μg/g wet weight whole brain tissue and are the average of at least three determinations ±s.e.

		Noradrenaline	Dopamine
Diluent treated controls		0·43 ± 0·03	0·78 ± 0·06
MBT	1 h	0·25 ± 0·01*	0·86 ± 0·02
	2 h	0·27 ± 0·02*	0·96 ± 0·03†
	4 h	0·42 ± 0·00	0·81 ± 0·02

* = $P < 0{\cdot}01$ † = $P < 0{\cdot}05$

Table 2. *Effect of* 2-*mercaptobenzothiazole* (MBT) *on the repletion of rat myocardial noradrenaline from exogenous dopamine after its depletion with metaraminol.* Rats were pretreated with metaraminol bitartrate, 5 mg/kg, 18 h before each received MBT, 300 mg/kg, or diluent. Dopamine hydrochloride, 35 mg/kg, or diluent was administered 30 min later and all rats were killed 3 h later. All values are expressed as μg/g and are the average of at least three determinations ±s.e.

	Myocardial noradrenaline
Diluent treated controls	0·88 ± 0·05
Metaraminol	0·15 ± 0·01
Metaraminol + dopamine	0·52 ± 0·09*
Metaraminol + MBT	0·18 ± 0·03
Metaraminol + MBT + dopamine	0·17 ± 0·02

* Significantly different from each of the other values, $P < 0{\cdot}05$.

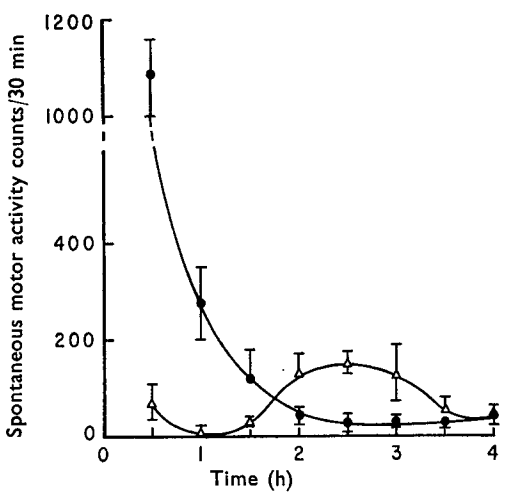

FIG. 1. Effect of 2-mercaptobenzothiazole, 300 mg/kg i.p., upon spontaneous motor activity in mice. Each point represents the average 30 min activity for six pairs of mice ± s.e. ——●——, control; ——△——, MBT.

4 h. Overtly, the treated mice were extremely depressed shortly after the administration of the drug and the depression accompanied by marked ptosis extended after 2 h. The return of amine levels to control concentrations paralleled a loss of overt depression. After 4 h the mice appeared normal.

We also treated rats with metaraminol bitartrate (5 mg/kg, i.p.) to deplete heart noradrenaline stores. After 18 h, rats were dosed with MBT (300 mg/kg) or diluent and 30 min later with dopamine (35 mg/kg). All rats were killed 3 h after the dopamine. The effect of MBT upon the repletion of rat myocardial noradrenaline from exogenous dopamine is summarized in Table 2. Exogenous dopamine restored the myocardial noradrenaline concentratations to 60% of the pre-drug level. At 300 mg/kg, MBT totally blocked the conversion of dopamine to newly synthesized noradrenaline. The effect of MBT upon spontaneous motor activity is shown in Fig. 1. Motor activity in control mice fell rapidly in the first hour and the mice remained relatively inactive for the remainder of the experiment. Initial exploratory activity was absent in MBT-treated mice. The increase in the spontaneous activity of treated mice at 2 h coincided with the termination of dopamine β-hydroxylase inhibitory activity in the brain as indicated by the recovery of brain noradrenaline stores between 2 and 4 h.

Thus, MBT effectively inhibits dopamine β-hydroxylase *in vitro* and *in vivo*. *In vitro*, MBT had 1/25th the inhibitory activity of disulfiram. *In vivo*, the compound altered mouse brain catecholamines in a manner consistent with that expected for inhibition of the enzyme in brain. In addition, the initial rate of decline of mouse brain noradrenaline ($0{\cdot}18\ \mu g/g/h^{-1}$) after MBT compares favourably with similar results we have obtained in mice after treatment with disulfiram and U-14,624 (Johnson & others, 1970). The effectiveness of the *in vivo* inhibition of noradrenaline synthesis by MBT was also clearly demonstrated by the complete block of the conversion of exogenous dopamine to noradrenaline in the rat myocardium. The recovery of both brain noradrenaline and dopamine to control levels by 4 h reflects a short term inhibition by MBT of mouse brain dopamine β-hydroxylase.

This compound should serve as an additional pharmacologic tool to study the effects of depleted noradrenaline levels concurrent with the short term *in vivo* inhibition of dopamine β-hydroxylase upon animal behaviour.

Metaraminol bitartrate was supplied by the Merck Institute for Therapeutic Research. The authors are very grateful to Mr. R. R. Russell for his technical assistance.

CNS Research,
The Upjohn Company,
Kalamazoo, Michigan 49001, *U.S.A.*

G. A. JOHNSON
S. J. BOUKMA
P. A. PLATZ

June 16, 1970

REFERENCES

ANTON, A. H. & SAYRE, D. F. (1962). *J. Pharmac. exp. Ther.*, **138**, 360–375.
EULER, U. S. von & FLODING, I. (1958). *Acta physiol. scand.*, **33**, *Suppl.*, 118, 45–46.
FRIEDMAN, S. & KAUFMAN, S. (1965). *J. biol. Chem.*, **240**, 4763–4773.
GOLDSTEIN, M., ANAGNOSTE, B. & LAUBER, E. (1964). *Life Sci.*, **3**, 763–767.
GOLDSTEIN, M., LAUBER, E. & MCKEREGHAN, M. R. (1964). *Biochem. Pharmac.*, **13**, 1103–1106.
GOLDSTEIN, M., PROCHOROFF, N. & SIRLIN, S. (1965). *Experientia*, **21**, 592–593.
JOHNSON, G. A., BOUKMA, S. J., & KIM, E. G. (1969). *J. Pharmac. exp. Ther.*, **168**, 229–234.
JOHNSON, G. A., BOUKMA, S. J. & KIM, E. G. (1970). *Ibid.*, **171**, 80–87.
JONSSON, J., GROBECKER, H. & GUNNE, L. M. (1967). *J. Pharm. Pharmac.*, **19**, 201–203.
NIKODIJEVIC, N., CREVELING, C. R. & UDENFRIEND, S. (1963). *J. Pharmac. exp. Ther.*, **140**, 224–228.
PALMER, J. K. & ROBERTS, J. B. (1967). *Science, N.Y.*, **157**, 200–201.
VELDKAMP, W., JOHNSON, G. A. & KEASLING, H. H. (1968). *J. pharm. Sci.*, **57**, 613–617.