J. Pharm. Pharmac., 1968, **20**, 605–609 Received January 15, 1968

Propranolol uptake into the central nervous system and the effect on rat behaviour and amine metabolism

R. LAVERTY AND K. M. TAYLOR

Propranolol readily penetrated all areas of the central nervous system after acute intraperitoneal administration and was cleared within 8–16 hr. Propranolol levels in the heart followed a similar pattern. After chronic oral administration brain levels of propranolol were equal to those 3 hr after intraperitoneal injection. Propranolol caused no significant difference in the brain levels of noradrenaline, normetanephrine, dopamine, 3-methoxytyramine, homovanillic acid, 5-hydroxytryptamine or total indoles, nor did it cause measurable changes in rat behaviour. An increase by propranolol in sleeping time after chloral hydrate in rats and mice was observed; this effect could not be correlated with changes in brain amine concentrations.

PROPRANOLOL in large doses has sedative and anti-convulsive properties in animals (Leszkovsky & Tardos, 1965); the central nervous activity does not seem to be related to the β-blocking activity of the drug (Murmann, Almirante & Saccani-Guelfi, 1966). It has been reported (Waal, 1967) that patients receiving propranolol have a high incidence of depression; this observation was not confirmed by Fitzgerald (1967). Brunner, Hedwell & others (1966) indicated that propranolol had a central nervous action and that treatment with propranolol for four days decreased the noradrenaline content of the rat brain and increased the heart noradrenaline content. However, Westfall (1967) reported that there were no significant changes in either heart or spleen noradrenaline following a range of doses of propranolol at 1 or 6 hr after treatment or after the daily administration of propranolol for 7 days.

Drugs causing depression and sedation may affect brain amine metabolism, so the uptake of propranolol by rat brain, and the effects of the drug administered acutely and chronically on catechol and indole amine metabolism and on behaviour have been examined.

Experimental

METHODS

For acute studies, propranolol (Inderal, I.C.I.) and control saline solutions were injected intraperitoneally into male albino rats (200–250 g). Propranolol was administered by two routes for chronic studies. It was given orally by replacing the rats' drinking water with a solution of 1% glucose and 0·5% sodium chloride containing propranolol (10 mg/100 ml). Ten male albino rats (150 g) received this fluid for 14 days, the daily intake being measured. Ten control rats received the solution not containing propranolol. In an attempt to duplicate the findings of Brunner & others (1966), their chronic administration method was repeated. Male albino rats (250 g) received daily subcutaneous injections of propranolol (10 mg/kg) for 4 days, and their brains were removed 2–2½ hr after the

From the Wellcome Medical Research Institute, University of Otago Medical School, Dunedin, New Zealand.

final injection. Control rats received normal saline injections. All doses are in terms of propranolol hydrochloride.

After the measured time the rats were decapitated, the brain, heart and other tissues removed, dissected, and immediately frozen over solid carbon dioxide and stored at −14° until estimated for the appropriate compound.

Determination of propranolol levels. The method of Stock & Westermann (1965) for the estimation of 1-(3-methylphenoxy) 3-isopropylaminopropanol (Kö 592) was adapted and proved more reliable and sensitive than the method of Black, Duncan & Shanks (1965). The tissue blank was equivalent to 0·05 μg/g of propranolol and a mean recovery of 77% (±3·3 s.e.; 11 determinations not corrected for extraction) was obtained. Tissue levels stated in terms of propranolol base are not corrected for recovery.

Determination of biogenic amines and metabolites. Noradrenaline, dopamine, normetanephrine and 3-methoxytyramine were determined on each brain sample by the hydroxyindole fluorimetric method (Taylor, 1967; Laverty & Taylor, 1967) using Dowex 50W cation exchange resin for amine separation (Bertler, Carlsson & Rosengren, 1958). Homovanillic acid was estimated by the fluorimetric method of Juorio, Sharman & Trajkov (1966). Mean recoveries (± s.e.) obtained were; for noradrenaline 89% (± 1·8; 14 values), dopamine 77% (±5·4; 12), normetanephrine 93% (±4·0; 2), 3-methoxytyramine 71% (±4·6; 2) and homovanillic acid 62% (±2; 10). Tissue levels of the above compounds are not corrected for recovery.

A modification of the method of Ashcroft & Sharman (1962) was used for 5-hydroxytryptamine and total indole determinations, the main alteration being the use of 0·4N perchloric acid as a protein precipitant. Mean recoveries were 5-hydroxytryptamine 63% (±4·2 s.e.; 13 values) and total indoles 53% (±7·6; 4 values); the tissue levels stated are not corrected for recovery.

Behavioural studies. The behaviour of albino and black male albino rats (200–250 g) after receiving intraperitoneal or oral propranolol was compared with that of control (saline-injected) rats using the following tests: (i) Y-runway of Steinberg, Rushton & Tinson (1961). (ii) Rotarod rotated at 7 and 12 rev/min. (iii) Conditioned avoidance response. (iv) Overnight activity in a modified jiggle cage.

Potentiation of sleeping time. Male mice (20–30 g) were tested for the potentiation of narcosis induced by chloral hydrate (Fastier, Speden & Waal, 1957) at a room temperature of 23°. A corresponding experiment using rats (200 g) at a chloral hydrate dose of 300 mg/kg was also made in a heated room at 29°. The concentration of noradrenaline, normetanephrine, dopamine and 3-methoxytyramine was determined in the thalamic regions and striatae of the treated rats.

Results

Propranolol levels. Table 1A indicates the brain and heart concentrations of propranolol after injection with 10 mg/kg intraperitoneally.

TABLE 1. PROPRANOLOL LEVELS IN RAT BRAIN AND HEART (μg/kg) AFTER INTRAPERITONEAL INJECTION OF PROPRANOLOL

A. 10 mg/kg

Time (hr)	Cortex			Thalamic regions			Striatae			Rhombencephalon			Whole brain			Heart		
	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range
0·25	2	3·29	2·64–3·49	–	—	—	–	—	—	–	—	—	–	—	—	2	3·79	3·69–3·88
0·5	3	2·77	2·53–2·99	2	2·74	2·33–3·15	2	3·49	3·48–3·50	2	2·02	2·00–2·05	2	2·59	2·54–2·64	1	1·85	—
1	6	2·24	1·65–2·62	4	2·04	1·65–2·44	4	3·08	2·78–3·38	4	1·45	1·23–1·67	4	2·07	1·90–2·24	3	0·89	0·70–1·26
1·5	1	1·99	—	–	—	—	–	—	—	–	—	—	–	—	—	2	0·90	0·87–0·93
2	6	1·12	0·66–1·56	2	0·88	0·82–0·93	2	1·33	1·09–1·57	2	0·51	0·40–0·62	2	0·79	0·67–2·24	4	0·73	0·60–0·86
3	6	0·44	0·26–0·94	4	0·42	0·48–0·63	4	0·52	0·37–0·66	6	0·33	0·12–0·46	4	0·31	0·31–0·47	–	—	—
4	–	—	—	–	—	—	–	—	—	–	—	—	–	—	—	2	0·38	—
6	2	0·14	0·13–0·15	2	0·25	0·17–0·32	2	0·29	0·23–0·34	2	0·11	0·08–0·13	2	0·17	—	2	0·15	—
8	–	—	—	–	—	—	–	—	—	–	—	—	–	—	—	2	0·00	—
16	6	0·00	—	6	0·00	—	6	0·00	—	6	0·00	—	6	0·00	—	2	0·00	—

B. 30 mg/kg

Time (hr)	Cortex		Spinal cord		Heart	
1	20·00	20·90	11·30	9·35	6·18	7·52
2	13·00	9·00	6·20	—	7·07	3·18
4	0·98	0·52	3·74	2·34	0·57	0·53
5	—	—	2·66	—	—	—
8	0·26	0·32	—	—	0·34	0·48

TABLE 2. LEVELS OF PROPRANOLOL, NORADRENALINE (NA), NORMETANEPHRINE (NM), DOPAMINE (DA), 3-METHOXYTYRAMINE (3MT), 5-HT, TOTAL INDOLES (5-OR) AND HOMOVANILLIC ACID (HVA) (μg/g) AFTER CHRONIC ORAL AND SUBCUTANEOUS ADMINISTRATION OF PROPRANOLOL

Treatment	Propranolol					NA	NM	DA	3MT	5-HT	5-OR	HVA
	Rhomb-encephalon	Thalamic regions	Striatae	Cortex	Whole brain	Thalamic regions + striatae (4 values) Mean (s.d.)				Thalamic regions (4 values) Mean (s.d.)		Striatae (4 values) Mean (s.d.)
14 days oral	0·20, 0·30	0·32, 0·21	0·67, 0·93	0·27, 0·23	0·32, 0·28	0·44 (0·02)	0·10 (0·04)	1·49 (0·20)	0·47 (0·14)	0·22 (0·07)	1·32 (0·09)	0·24 (0·04)
Control	—	—	—	—	—	0·46 (0·03)	0·10 (0·02)	1·46 (0·18)	0·36 (0·08)	0·20 (0·07)	1·42 (0·10)	0·25 (0·04)
						Whole brain (8 values) mean (s.d.)						
4 days S.C. 10 mg/kg ..	—	—	—	—	—	0·32 (0·03)	0·06 (0·01)	0·51 (0·04)	0·13 (0·05)			
Control	—	—	—	—	—	0·33 (0·02)	0·05 (0·01)	0·54 (0·04)	0·22 (0·09)			

It will be seen that propranolol can be detected in both central nervous and heart tissue for a period of 8–16 hr after a single intraperitoneal injection. During chronic oral administration, the mean intake of propranolol was 4·25 mg/day/rat over the 14-day period. The brain propranolol levels in these rats, when killed, showed a similar distribution to that found after an intraperitoneal injection, though the levels in the striate region were perhaps relatively higher.

Catecholamine, 5-hydroxytryptamine and metabolite levels. In 21 experiments using over 180 rats, no change was found in the content in whole brain or in various brain regions of noradrenaline, normetanephrine, dopamine, 3-methoxytyramine, homovanillic acid, 5-hydroxytryptamine or total 5-OR indoles at $\frac{1}{4}$ to 16 hr after the intraperitoneal injection of propranolol at 10 and 30 mg/kg. After chronic oral and subcutaneous treatment (Table 2), no change in content of amines or metabolites was found.

Behavioural studies. Despite extensive studies using the Y-runway, rotarod, conditioned avoidance response and overnight activity apparatus, no change in behaviour due to propranolol was detected following acute or chronic administration by injection or oral administration.

Potentiation of sleeping time. The duration of sleeping following chloral hydrate was increased by propranolol in both rats and mice. Six saline-injected rats slept 34·5 $\pm$ 4·4 min (mean $\pm$ s.e.) whereas equal groups of propranolol-injected rats slept 57·3 $\pm$ 4·4 min after 10 mg/kg and 75·6 $\pm$ 4·7 min after 30 mg/kg; these were significantly different from the saline-injected group at $P < 0{\cdot}01$, $P < 0{\cdot}001$ respectively. In three groups of 18 mice similarly treated, the sleeping times in minutes were 24·0 $\pm$ 0·5 for controls, 25·4 $\pm$ 0·7 for 10 mg/kg and 61·6 $\pm$ 2·1 after 30 mg/kg; this last value was significantly different from the saline-injected group ($P < 0{\cdot}001$). Brain levels of noradrenaline, normetanephrine, dopamine and 3-methoxytyramine were not changed in propranolol-treated rats compared with controls also treated with chloral hydrate.

Discussion

Propranolol readily penetrates the blood-brain barrier to all areas of the rat brain (Table 1) and is cleared from nervous tissue, as from the heart, relatively quickly, tissue levels having returned to zero 8–16 hr after a single intraperitoneal injection. After chronic oral administration at a dose rate of approximately 20 mg/kg/day (Table 2), the brain tissue content of propranolol corresponded to that found 3–4 hr after a single intraperitoneal injection of 10 mg/kg.

Propranolol prolongs the hypnotic effect of chloral hydrate in both rats and mice; these results extend and confirm those of Leszkovsky & Tardos (1965) and Murmann & others (1966) who studied the potentiation of barbiturate anaesthesia. Hence propranolol may have some action on the central nervous system, though no effects of propranolol on various other behavioural measurements or on brain amine metabolism were observed at the dose levels used. Brunner & others (1966) had reported

a fall in brain noradrenaline and an increase in heart noradrenaline after repeated propranolol administration; we could not repeat this observation using identical dose and time schedules. Westfall (1967) also reported no effect on tissue noradrenaline content after acute and repeated propranolol administration.

Since in the present experiments in rats there was no change in observable behaviour or in amine metabolism, it is not possible to predict whether or not the reported propranolol-induced depression in humans is associated with changes in brain amine metabolism.

Acknowledgements. This work was supported by a grant from the Golden Kiwi Medical Research Distribution Committee. We are grateful to Dr. C. R. Proctor, I.C.I.A.N.Z., Melbourne, for supplies of propranolol, and Mr. P. Arnott for skilled technical assistance.

References

Ashcroft, G. W. & Sharman, D. F. (1962). *Br. J. Pharmac. Chemother.*, **19**, 153–160.
Bertler, Å., Carlsson, A. & Rosengren, E. (1958). *Acta physiol. scand.*, **44**, 273–292.
Black, J. W., Duncan, W. A. M. & Shanks, R. G. (1965). *Br. J. Pharmac. Chemother.*, **25**, 577–591.
Brunner, H., Hedwell, P., Maître, L. & Meier, M. (1966). *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **254**, 45–55.
Fastier, F. N., Speden, R. N. & Waal, H. (1957). *Br. J. Pharmac. Chemother.*, **12**, 251–256.
Fitzgerald, J. D. (1967). *Br. med. J.*, **2**, 372–373.
Juorio, A. V., Sharman, D. F. & Trajkov, T. (1966). *Ibid.*, **26**, 385–392.
Laverty, R. & Taylor, K. M. (1967). *Proc. Univ. Otago med. Sch.*, **45**, 8–11.
Leszkovsky, G. & Tardos, L. (1965). *J. Pharm. Pharmac.*, **17**, 518–519.
Murmann, W., Almirante, L. & Saccani-Guelfi, M. (1966). *Ibid.*, **18**, 317–318.
Steinberg, H., Rushton, R. & Tinson, C. (1961). *Nature, Lond.*, **192**, 533–535.
Stock, K. & Westermann, E. (1965). *Biochem. pharmac.*, **14**, 227–236.
Taylor, K. M. (1967). M. Pharm. Thesis, University of Otago, New Zealand.
Waal, H. (1967). *Br. med. J.*, **2**, 50.
Westfall, T. C. (1967). *Archs int. Pharmacodyn. Thér.*, **167**, 69–79.