The influence of neuroleptics on amphetamine metabolism in the rat and guinea-pig*

ROBERT A. YOKEL, *Department of Pharmacology, University of Cincinnati Medical Center, Cincinnati, Ohio* 45267, *U.S.A.*

Many of the effects of amphetamine, including hyperthermia, central nervous system stimulation, hypertension, tachycardia, and psychosis can be attenuated by neuroleptic drugs (Lemberger, Witt & others, 1970; Hill & Horita, 1971; Snyder, 1973; Angrist, Lee & Gershon, 1974). Haloperidol has been shown to reduce the incidence of amphetamine lethality in experimental animals (Davis, Logston & Hickenbottom, 1974; Catravas, Waters & others, 1975). The only neuroleptic extensively tested in amphetamine poisoning in man has been chlorpromazine, which was recommended as an amphetamine antidote (Espelin & Done, 1968).

A neuroleptic, to be of practical value in the treatment of amphetamine toxicity in man, should not inhibit the metabolism of amphetamine. In man, amphetamine is hydroxylated in the *para*-position (3–7%) to less active compounds, deaminated (15–29%) to inactive metabolites, and excreted unchanged in the urine (19–30%, when the urine pH is not controlled) (Dring, Smith & Williams, 1970; Caldwell, Dring & Williams, 1972; Sever, Caldwell & others, 1973). Chlorpromazine is known to inhibit amphetamine metabolism in the rat (Lemberger & others, 1970; Lal, Sourkes & Missala, 1974).

To determine if other neuroleptic drugs influence amphetamine metabolism, the influence of various neuroleptic drugs on amphetamine brain concentration was tested in the rat and guinea-pig. The rat was chosen as a species in which the major route of metabolism is hydroxylation (43–61%), together with deamination (3–6%) and the excretion of some amphetamine unchanged in the urine (13–31%) (Dring & others, 1970). The guinea-pig was chosen because it deaminates (45–62%) amphetamine, and excretes some (22–55%) unchanged, but does not hydroxylate it (Dring & others, 1970; Lewander, 1971). Neuroleptics were chosen from each of the major classes available.

Male Sprague Dawley rats (Laboratory Supply Company, Indianapolis) and male Hartley guinea-pigs (Sweetwater Farm, Hillsboro, Ohio) 250–300 g were used. Animals were injected intraperitoneally with [^{14}C](+)-amphetamine sulphate, 2 mg kg^{-1} (1 μCi mg^{-1}) in saline. [^{14}C](+)-Amphetamine was obtained from Smith, Kline, and French. Fifteen min later, 12 animals of each species were injected intraperitoneally with one of the seven neuroleptics listed in Table 1 or with 0·9% NaCl. The neuroleptic doses used are equivalent clinically (Goodman & Gilman, 1975), or experimentally in attenuating amphetamine activity (Janssen, Niemegeers & others, 1968; Voith & Herr, 1975).

* Supported by a grant from the University Research Council University of Cincinnati.

Table 1. *Neuroleptics tested for their effects on amphetamine disappearance from brains of rats and guinea-pigs, and the results obtained.*

Neuroleptic	Dose (mg kg^{-1})	Preparation	Amphetamine half-life ± s.e. (min) Rat	Guinea-pig
Control	—	normal saline	48 ± 2	121 ± 32
Chlorpromazine	15	commercial injectable solution	222 ± 61	266 ± 67
Perphenazine	2	in 0·03M HCl	70 ± 6	114 ± 23
Thioridazine	15	in water	590 ± 665	123 ± 22
Thiothixene	1	in water with 3·3% Tween 80	55 ± 4	127 ± 19
Haloperidol	1	in water	61 ± 6	131 ± 21
Pimozide	5	in water with 6 part tartaric acid : 1 part pimozide	68 ± 8	183 ± 60
(+)-Butaclamol	1	in water	57 ± 5	128 ± 38

Four animals in each group were decapitated 1, 2, or 4 h after the second injection. The portion of the brain anterior to the pons was removed and frozen until extraction of the amphetamine was conducted. The brains were later homogenized in a Ten Broeck Vitro '200' Tissue Grinder in 4 parts of 0·01M HCl. [^{14}C](+)-Amphetamine was extracted from the homogenized tissue by a modification of the procedure which extracts amphetamine from its metabolites (Axelrod, 1954). Twelve ml benzene and 2 ml 2M NaOH were added to tubes containing 2 ml of homogenized tissue. The tubes were shaken for 5 min, centrifuged and the aqueous phase removed and discarded. Following the addition of 2·5 ml 0·5M NaOH, the tubes were shaken for 5 min, centrifuged, and 10 ml of the organic phase was removed to another tube to which 1 ml 1M HCl was added. The tubes were shaken, centrifuged, and 0·9 ml of the acid phase transferred to scintillation vials containing 12 ml Insta-Gel (Packard Instrument). Radioactivity was measured in a Packard Tri-Carb Model 3320 scintillation spectrometer.

Mean brain amphetamine concentrations for groups of four animals decapitated at 1, 2, or 4 h after receiving saline or neuroleptic injection are shown in Table 2. Chlorpromazine delayed the disappearance from brain of amphetamine in both the rat and guinea-pig and thioridazine did so in the rat. No other neuroleptics

Table 2. *Amphetamine brain concentrations ($\mu g\ g^{-1}$ brain tissue) after saline or neuroleptic injection.* Mean of four animals and coefficient of variation (in parentheses).

Drug mg kg^{-1}	Time (h) after neuroleptic injection		
Rat	1	2	5
Saline	937 (18%)	373 (16%)	68 (20%)
Chlorpromazine 15	1908 (15%)	1394 (14%)	1098 (36%)
Perphenazine 2	670 (5%)	399 (15%)	121 (40%)
Thioridazine 15	1160 (35%)	1483 (21%)	965 (23%)
Thiothixene 1	803 (7%)	495 (27%)	92 (26%)
Haloperidol 1	881 (35%)	321 (18%)	102 (16%)
Pimozide 5	938 (45%)	499 (19%)	144 (25%)
(+)-Butaclamol 1	1201 (12%)	487 (21%)	136 (40%)
Guinea-pig			
Saline	1367 (16%)	690 (16%)	508 (20%)
Chlorpromazine 15	1187 (7%)	1225 (5%)	787 (23%)
Perphenazine 2	1077 (13%)	902 (21%)	405 (47%)
Thioridazine 15	1136 (26%)	790 (28%)	411 (26%)
Thiothixene 1	1278 (16%)	880 (32%)	471 (16%)
Haloperidol 1	1414 (11%)	967 (30%)	546 (23%)
Pimozide 5	1141 (6%)	747 (36%)	575 (32%)
(+)-Butaclamol 1	1207 (2%)	848 (23%)	525 (50%)

appreciably influenced amphetamine brain concentrations. The half-life of amphetamine in brain after saline or neuroleptic injection for rat and guinea-pig are shown in Table 1. Half-lives were calculated from the line of best fit.

The results reported herein confirm and extend previous findings on the influence of neuroleptics on amphetamine disappearance from the brain. Chlorpromazine was found to inhibit amphetamine disappearance from rat brain, as previously reported (Lemberger & others, 1970; Lal & others, 1974), and was also found to inhibit amphetamine disappearance from guinea-pig brain. Showing the results from one patient, Lemberger (1972) reported that during a course of chlorpromazine therapy, amphetamine disappearance was not affected, however, the doses of chlorpromazine and amphetamine were not reported.

As previously reported, pimozide, perphenazine, and haloperidol in doses used in the present study did not inhibit amphetamine disappearance from the rat (Lewander, 1969; Lemberger & others, 1970; Lal, Missala & Sourkes, 1971; Soudijn & van Wijngaarden, 1972), although higher doses of perphenazine and haloperidol have been shown to inhibit amphetamine disappearance (Lemberger & others, 1970; Lal & others, 1974). The control brain half-lives of 48 min for the rat and 121 min for the guinea-pig agree well with previous determinations (Stolk & Rech, 1969; Lemberger & others, 1970; Lewander, 1971; Lal & others, 1974).

The present study differs from previously reported studies in that the neuroleptic injections were given after amphetamine, rather than before, making the results reported here potentially more significant clinically. The results obtained suggest that chlorpromazine and thioridazine, due to their ability to delay the disappearance of amphetamine from the brain of the rat and guinea-pig, may not be the agents of choice in the treatment of human amphetamine poisoning. Perhaps one of the other neuoleptics, such, as haloperidol, would be a more prudent choice.

April 25, 1978

REFERENCES

ANGRIST, B., LEE, H. K. & GERSHON, S. (1974). *Am. J. Psychiat.*, **131**, 817–819.

AXELROD, J. (1954). *J. Pharmac. exp. Ther.*, **110**, 315–326.

CALDWELL, J., DRING, L. G. & WILLIAMS, R. T. (1972). *Biochem. J.*, **129**, 23–24.

CATRAVAS, J. D., WATERS, I. W., DAVIS, W. M. & HICKENBOTTOM, J. P. (1975). *J. Am. med. Ass.*, **231**, 1340–1341.

DAVIS, W. M., LOGSTON, D. G. & HICKENBOTTOM, J. P. (1974). *Tox. appl. Pharmac.*, **29**, 397–403.

DRING, L. G., SMITH, R. L. & WILLIAMS, R. T. (1970). *Biochem. J.*, **116**, 425–435.

ESPELIN, D. E. & DONE, A. K. (1968). *New Engl. J. Med.*, **278**, 1361–1365.

GOODMAN, L. S. & GILMAN, A. (1975). *The Pharmacological Basis of Therapeutics*, 5th edn., p. 170. New York: MacMillan Co.

HILL, H. F. & HORITA, A. (1971). *J. Pharm. Pharmac.*, **23**, 715–717.

JANSSEN, P. A. J., NIEMEGEERS, G. E., SCHELLEBENS, K. H. L., DRESSE, A., LENAERTS, F. M., PINCHARD, A., SCHAPER W. K. A., VAN NUETEN, J. M. & VERBRUGGEN, F. J. (1968). *Arzneimittel-Forsch.*, **18**, 261–279.

LAL, S., MISSALA, K. & SOURKES, T. (1971). *J. Pharm. Pharmac.*, **23**, 967–969.

LAL, S., SOURKES, T. L. & MISSALA, K. (1974). *Archs int. Pharmacodyn. Thér.*, **207**, 122–130.

LEMBERGER, L., WITT, E. D., DAVIS, J. M. & KOPIN, I. J. (1970). *J. Pharmac. exp. Ther.*, **174**, 428–433.

LEMBERGER, L. (1972). *J. pharm. Sci.*, **61**, 1690–1694.

LEWANDER, T. (1969). *Eur. J. Pharmac.*, **6**, 38–44.

LEWANDER, T. (1971). *Acta Pharm. Tox.*, **29**, 209–225.

SEVER, P. S., CALDWELL, J., DRING, L. G. & WILLIAMS, R. T. (1973). *Eur. J. Clin. Pharmac.*, **6**, 177–180.

SNYDER, S. H. (1973). *Am. J. Psychiat.*, **130**, 61–67.

SOUDIJN, W. & VAN WIJNGAARDEN, I. (1972). *J. Pharm. Pharmac.*, **24**, 773–780.
STOLK, J. M. & RECH, R. H. (1969). *Biochem. Pharmac.*, **18**, 2786–2788.
VOITH, K. & HERR, F. (1975). *Psychopharmacologia*, **42**, 11–20.

A possible augmentation of the extraneuronal adrenaline uptake caused by inhibition of the neuronal component

T. KATSURAGI*, *Department of Applied Physiology, Tohoku University School of Medicine, Seiryo-cho* 2-1, *Sendai*, 980 *Japan*

It is well known that when a high concentration of a catecholamine such as adrenaline is applied to adrenergically-innervated preparations, the amine is rapidly taken up into both the neuronal ($Uptake_1$) and extraneuronal ($Uptake_2$) sites (Iversen, 1965; Gillespie & Hamilton, 1966; Draskóczy & Trendelenburg, 1970). The manner of the amine uptake into the extraneuronal site differs from that into the neuronal site in many respects, such as energy dependency and sensitivity to various uptake inhibitors, but the precise mechanism involved remains obscure. In the present experiments, an attempt has been made to determine whether the amount of adrenaline taken up into the extraneuronal compartment is altered when transport across the axonal membrane is inhibited.

There is clear-cut evidence (Hertting, 1964; Callingham & Burgen, 1966; Ross & Renyi, 1966) that isoprenaline can be taken up into the extraneuronal, but not into the neuronal site. Thus, the transport of this amine under the conditions described above has also been examined in comparison with adrenaline, which is capable of being taken up into both sites (Iversen, 1965; Draskóczy & Trendelenburg, 1970).

The vas deferens isolated from a male guinea-pig was suspended in a bath (1 ml) containing a tris buffered saline solution pH 7·4 described by Paton (1973) and bubbled with O_2. Throughout the experiment, the bathing medium was maintained at approx. 37°. After equilibration for 1 h, the preparation was loaded with (±)-adrenaline or (±)-isoprenaline (3×10^{-5} M) for 15 min, rapidly washed three times with amine-free medium, and then kept for 20 min in this medium. This washout period was used since previous results (Bönisch, Uhlig & Trendelenburg, 1972; Lindmar & Löffelholz, 1972; Katsuragi & Suzuki, 1976) have shown that the spontaneous release of the total extraneuronal amine occurs long before that of the neuronal amine. The amounts of the catecholamine released into the final washout medium were fluorimetrically determined by the trihydroxyindole method at pH 3·5 and pH 7·0 as described by Katsuragi & Suzuki (1977). The values of the amines, determined at pH 3·5 to avoid contamination from the endogenous noradrenaline, are shown in Figs 1 and 2. Also, the mandelic acid metabolites of adrenaline, produced by monoamine oxidase, did not affect the adrenaline fluorescence, while any endogenous metanephrine, produced by catechol-*O*-methyltransferase, was discriminated from adrenaline by the method used in a previous study (Katsuragi & Suzuki, 1977). Adrenaline and isoprenaline were measured at 335/510 and 330/500 nm, respectively. It has been assumed that the amount of amine found in both fluids after 20 min is a measure of the amount of uptake into the extraneuronal tissue of the vas deferens.

As shown in Fig. 1, surgical denervation (1 week previously; Birmingham, 1970) or addition 10 min before loading with amine or cocaine (10^{-5} M), desipramine ($3{\cdot}8 \times 10^{-5}$ M) or ouabain (10^{-4} M), which inhibit neuronal amine uptake, greatly enhanced the spontane-

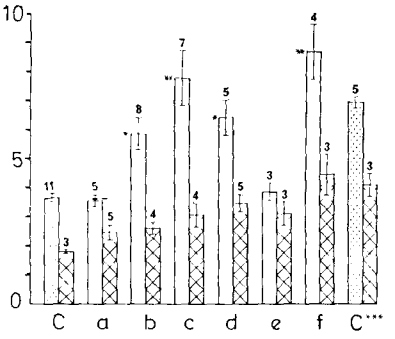

FIG. 1. Effects of blockade of neuronal amine uptake on the efflux of adrenaline output from extraneuronal-compartments in guinea-pig vas deferens. Drugs, including clonidine ($1{\cdot}9 \times 10^{-4}$ M), but not reserpine, were added to the bath 10 min before the loading of adrenaline. Values of adrenaline uptake are shown by amounts (n mol g^{-1} wet weight of tissue) (ordinate) of spontaneous release of the amine in the bath throughout the washout period. Vertical bars: mean ±s.e. Number in parentheses represent the number of experiments. Control***: loading with 9×10^{-5} M adrenaline. ** Differ from control, $P < 0{\cdot}01$; * differ from control, $P < 0{\cdot}05$. C: Control; a: reserpine; b: denervation; c: cocaine (10^{-5} M); d: DMI ($3{\cdot}8 \times 10^{-5}$ M); e; ouabain (10^{-5} M); f: ouabain (10^{-4} M). Cross-hatched columns show pretreatment with clonidine. Stippled columns show control uptake with clonidine.

*Present address: Department of Pharmacology, School of Medicine, Fukuoka University, Nanakuma 34, Fukuoka, 814 Japan.