OPERANT BEHAVIOURAL DEMONSTRATION OF QUALITATIVE DIFFERENCES BETWEEN THE *d*- AND *l*-ISOMERS OF AMPHETAMINE*

S. B. SPARBER and D. W. PETERSON
Department of Pharmacology and Psychiatry Research Unit of the Department of Psychiatry, University of Minnesota, Minneapolis, Minnesota 55455, U.S.A.

MOST of the the literature on the pharmacology of amphetamine indicates that the *d* and *l* isomers are about equiactive in their effects upon measures reflective of responses by peripheral organs (ALLES, 1939; VAN LIERE, *et al.*, 1951; DAY, 1965; RUDZIK and EBLE, 1967; CLAY, *et al.*, 1971; SCHNEIDER, 1972), while the best estimate of their potencies upon behavioural variables or reversal of sedation or depression induced by other drugs indicates that *d*-amphetamine is at least 2–4 times more active than *l*-amphetamine (ALLES, 1939; PRINZMETAL and ALLES, 1940; SCHULTE, *et al.*, 1941; DEWHURST and MARLEY, 1965; MAJ, *et al.*, 1972; WALLACH and GERSHON, 1972). Although implicit in the above statement is their effects upon the central nervous system, there is evidence that peripheral tissue, alone or in combination with a central component, may also show a greater potency for the *d*- over the *l*-isomer. (BLASCHKO and STROMBLAD, 1960; BHARGAVA, *et al.*, 1963; BURGEN and IVERSON, 1965; DALY, *et al.*, 1966; MALING, *et al.*, 1972; SCHNEIDER, 1972).

Although the neurochemical studies purporting to show regional differences in the degree to which the two isomers inhibit the uptake or facilitate the release of norepinephrine (NE) and dopamine (DM) are equivocal, (TAYLOR and SNYDER, 1970; FERRIS, *et al.*, 1972), the differences appear not whether or not a potency ratio different from 1 exists between *d* and *l*-amphetamine but where in the CNS this difference emerges. In addition, SVENSSON (1971) reported that along with an increase in locomotor activity produced by both isomers, *l*-amphetamine increased DM levels and decreased NE levels while *d*-amphetamine decreased both catecholamines in mouse brain.

One of the major problems associated with studying the behavioural and/or neurochemical consequences of pharmacological agents is that no one unitary hypothesis can describe the mechanism by which drugs act. For example, to assume a common mechanism because patterns or levels of unconditioned or conditioned behaviour are 'identically' affected by two different drugs, would be highly questionable. Why then must we assume that isomers of the same drug act through the same mechanism but are only quantitatively different, if more of one isomer is necessary to produce the 'same effect' as the other isomer, especially in light of neurochemical evidence to the contrary.

* Supported in part by grants from the U.S.P.H.S. MH 08565, DA 00532 and GM 01117.

We have been addressing ourselves to methodological as well as theoretical problems of this type during the past several years and some of our data support the possibility that *d* and *l*-amphetamine, in addition to or aside from being quantitatively different, are also qualitatively different regarding the mechanism by which they affect certain classes of operant behaviour.

We have gone about this in several ways and I would like to discuss the advantages and limitations of our methods. In choosing a more controllable behaviour measure we have focussed upon operant techniques. However, like other measures of behaviour, the schedule of reinforcement and history of the organism can generate fairly resistant behaviour or labile behaviour which is more easily disrupted or otherwise altered by drug administration (KELLEHER and MORSE, 1969). Fixed ratio behaviour is quite stable and requires relatively greater doses of drug to disrupt. On the other hand, schedules which generate lower or moderate rates of responding appear to be more sensitive for measuring drug effects, often demonstrating rate-increasing effects prior to disruption. We have attempted to use these principles as well as those of tolerance and cross-tolerance to assess isomeric differences.

When a dose of 1 mg *d*-amphetamine sulfate/kg is administered (i.p.) to rats trained to bar-press for food pellets on a fixed ratio 30 (FR-30) schedule, the behaviour is altered so that the subjects are responding at about 40% of control rates. A dose of 4 mg l-amphetamine sulfate/kg likewise brought FR-30 responding down to about 40% of control. However, when tolerance to the disruptive effect of either isomer (at equieffective doses) developed, a challenge by the other isomer resulted in a demonstration of no cross-tolerance, suggesting differential mechanisms (TILSON and SPARBER, in press). If a more sensitive measure of operant behaviour is used, we see a differential effect of *d* and *l*-amphetamine on components of fixed-interval (FI-75 seconds) responding. Whereas low doses of *d*-amphetamine (0·16 and 0·50 mg/kg) increase responding early in the interval as well as late in the interval, resulting in an overall increase in FI behaviour, *l*-amphetamine increases responding late in the interval at 0·5 and 1·0 mg/kg. Overall FI behaviour is likewise increased slightly (beyond 2 SD of control values) but the pattern or distribution of responding among the segments of the FI is not the same. If one had to choose equieffective rate-increasing doses of *d* and *l*-amphetamine, the best approximation would be 0·16 mg/kg of the *d*-isomer and 0·50 mg/kg of the *l*-isomer, again the 1:3 ratio. However, after tolerance to the rate-increasing effects of either isomer, challenge the next session by the opposite isomer resulted in a drug effect or lack of cross tolerance (TILSON and SPARBER, in press).

Injections of 6-hydroxydopamine into the lateral ventricle of rats, at dosages that have little or no effect upon DM in various regions of brain but significantly lower NE concentrations, increase overall FR-responding significantly as long as 155 days after 6-OHDM. However, while the dose-response curves for *d*-amphetamine are virtually identical for both treated and control groups, (PETERSON and SPARBER, unpublished) the curves for *l*-amphetamine are not, the 6-OHDM treated rats being more resistant to the disruptive action of *l*-amphetamine (Fig. 1).

One interpretation of these last data might be a demonstration of the importance of DM or lack of relative importance of NE for at least one effect of *d*-amphetamine. Alternatively, it might indicate a relatively greater importance of NE for the action of *l*-amphetamine. In any event, the general conclusion we have come to is that in

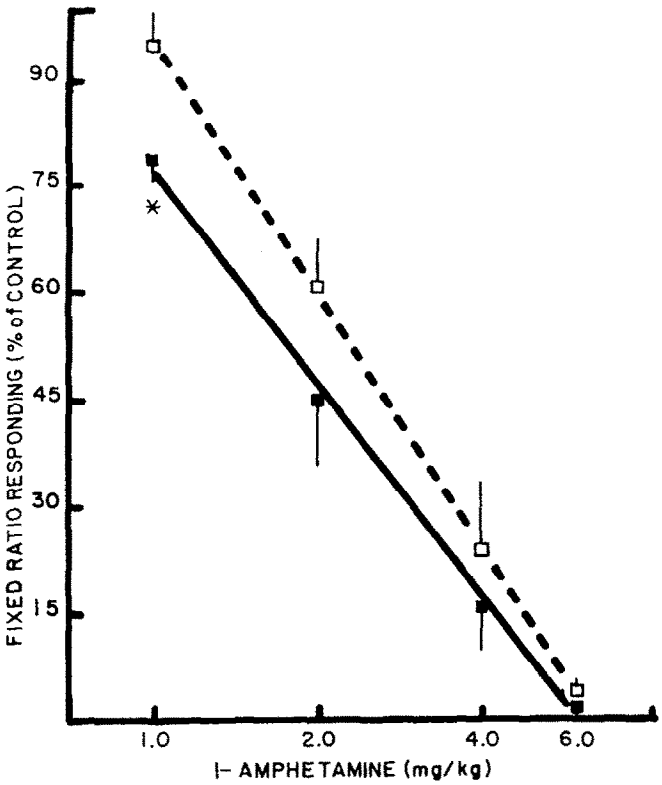

FIG. 1.—Log dose response curves demonstrating significant ($P < 025$) differences between intraventricularly 6-OHDM (□) treated rats and vehicle controls (■) in their responsiveness to the FR-30 disruptive action of L-amphetamine. Animals were injected via their right lateral ventricles with either 200 μg 6-OHDM/15 μl of 0·1% ascorbic acid in saline or vehicle. L-Amphetamine's effect was determined, along with that of *d*-amphetamine, between 30 and 72 days after treatment. Data is derived for nine rats in each group, using saline injection as the control one day before each dose of *l*-amphetamine. Vertical lines at each point show 1 S.E.M. Data was analyzed according to analysis of variance for bioassay curves and Student's t-test.

addition to differential neurochemical actions described *in vitro*, with appropriate tests, behavioural differences of a qualitative nature likewise emerge.

REFERENCES

ALLES G. A. (1939). Comparative actions of optically isomeric phenisopropylamines. *Am. J. Physiol.* **126,** P 420.

BHARGAVA K. P., KAR K. and PARMAR S. S. (1963). Effects of enzyme inhibitors on the adrenergic mechanisms in isolated vas deferens preparation. *Life Sci.* **2,** 989–992.

BLASCHKO H. and STROMBLAD B. C. R. (1960). The inhibition of human amine oxidase by the two isomers of amphetamine. *Arzneim. Forsch.* **10,** 327.

BURGEN A. S. V. and IVERSON L. L. (1965). The inhibition of noradrenaline uptake by sympathomimetic amines in the rat isolated heart. *Br. J. Pharmacol.* **25,** 34–49.

CLAY G. A., CHO A. K. and ROBERFROID M. (1971). Effect of diethylaminoethyl diphenylpropylacetate hydrochloride (SKF-525A) on the norepinephrine-depleting actions of *d*-amphetamine. *Biochem. Pharmacol.* **20,** 1821–1831.

DALY J. W., CREVELING C. R. and WITKOP B. (1966). The chemorelease of norepinephrine from mouse hearts. Structure-activity relationships. I. Sympathomimetic and related amines. *J. Med. Chem.* **9,** 273–284.

DAY M. D. (1965). Influence of the length of the stimulus period and frequency of sympathetic stimulation on the response of the guinea-pig isolated vas deferens to bretylium, guanethidine and amphetamine. *J. Pharm. Pharmacol.* **17**, 619–627.

DEWHURST W. G. and MARLEY E. (1965). The effects of α-methyl derivatives of noradrenaline, phenylethylamine and tryptamine on the central nervous system of the chicken. *Br. J. Pharmacol.* **25**, 682–704.

FERRIS R. M., TANG F. L. M. and MAXWELL R. A. (1972). A comparison of the capacities of isomers of amphetamine, deoxypipradrol and methylphenidate to inhibit the uptake of tritiated catecholamines into rat cerebral cortex slices, synaptosomal preparations of rat cerebral cortex, hypothalamus and striatum and into adrenergic nerves of rabbit aorta. *J. Pharmacol. Exp. Ther.* **181**, 407–416.

KELLEHER R. T. and MORSE W. H. (1968). Determinants of the specificity of behavioral effects of drugs. *Ergbn. der. Physiol.* **60**, 1–56.

MAJ J., GRABOWSKI M., GAJDA L. and MICHALUK, J. (1972). Pharmacologic action of d and l-amphetamine. *Dissert. Pharm. Pharmacol.* **24**, 7–16.

PRINZMETAL M. and ALLES G. A. (1940). The central nervous system stimulant effects of dextro-amphetamine sulfate. *Am. J. Med. Sci.* **200**, 665–673.

RUDZIK A. D. and EBLE J. N. (1967). The potentiation of pressor responses to tyramine by a number of amphetamine-like compounds. *Proc. Soc. Eptl. Biol. Med.* **124**, 655–657.

SCHNEIDER F. H. (1972). Amphetamine-induced exocytosis of catecholamines from the cow adrenal medulla. *J. Pharmacol. Exp. Ther.*, **183**, 80–89.

SCHULTE J. W., REIF E. C., BACHER J. A., LAWRENCE W. S. and TAINTER M. L. (1941). Further study of central stimulation from sympathomimetic amines. *J. Pharmacol. Exp. Ther.* **71**, 62–74.

SVENSSON T. H. (1971). Functional and biochemical effects of d- and l-amphetamine on central catecholamine neurons, *Naunyn-Schmiederbergs Arch. Pharmak.* **271**, 170–180.

TAYLOR K. M. and SNYDER S. H. (1970). Amphetamine: Differentation by d and l-isomers of behavior involving brain norepinephrine or dopamine. *Science* **168**, 1487–1489.

TILSON H. A. and SPARBER S. B. (in press). The effects of *d*- and *l*-amphetamine on fixed-interval and fixed-ratio behavior in tolerant and non-tolerant rats. *J. Pharmacol. Exp. Ther.*

VAN LIERE E. J., STICKNEY J. C., NORTHRUP D. W. and BELL R. O. (1951). Effect of dl-amphetamine sulfate and its isomers on intestinal mobility. *J. Pharamcol. Exp. Ther.* **103**, 187–189.

WALLACH M. B. and GERSHON S. (1972). The induction and antagonism of central nervous system stimulant-induced stereotyped behavior in the cat. *Eur. J. Pharmacol.* **18**, 22–26.