J. Pharm. Pharmac., 1978, **30**, 88–91 *Received July* 4, 1977

Effect of tolerance to morphine, ethanol and barbiturate on amphetamine circling in rats with a striatal dopamine lesion

P. SLATER

Department of Physiology, The Medical School, University of Manchester, Manchester M13 9PT, U.K.

The (+)-amphetamine circling rate of rats with unilateral 6-OHDA lesions in the striatum was recorded. Morphine tablets were implanted subcutaneously for chronic treatment. In the morphine-dependent animal the circling rate to amphetamine given 4 days after morphine was first implanted was depressed but after withdrawal with naloxone a day later the rate increased, returning to normal after 21 days. Barbiturate physical dependence was induced by adding increasing amounts of barbitone to the drinking water of lesioned rats over four weeks after which the amphetamine circling response was depressed and remained so after the barbiturate was withdrawn. Ethanol tolerance was induced by adding ethanol to the drinking water of lesioned rats for four weeks. Neither the induction of tolerance over this period nor ethanol withdrawal had any effect on the circling response to amphetamine. The change in the response of striatal dopamine neurons to amphetamine that occurs after chronic morphine treatment, cannot be produced by chronic treatment with either barbitone or ethanol. The neurochemical bases of barbiturate and ethanol tolerance are different from morphine tolerance.

The rat with a unilateral 6-hydroxydopamine (6-OHDA) lesion of the nigrostriatal dopamine projection (Ungerstedt, 1971) is used for investigating the effect of a drug on brain dopamine neurons. Morphine antagonizes the circling produced in such lesioned rats by apomorphine, a dopamine receptor agonist, and by (+)-amphetamine, which releases endogenous dopamine (Blundell, Crossman & Slater, 1976). Because morphine was more effective against amphetamine, it was suggested that, as well as blocking dopamine receptors, morphine might also suppress dopamine release.

There are three classes of drug which produce tolerance and physical dependence. These are the opiates, the barbiturates and ethanol. Chronic treatment with morphine affects dopamine neurons involved in thermo-regulation (Cox, Ary & Lomax, 1976). Although ethanol has no effect on brain dopamine content (Efron & Gessa, 1963; Duritz & Truitt, 1966), chronic ethanol treatment causes changes in dopamine turnover and release (Hunt & Majchrowicz, 1974; Darden & Hunt, 1975; Karoum, Wyatt & Majchrowicz, 1976).

The circling rat model has now been used to examine the effects of morphine tolerance and physical dependence on amphetamine-induced circling. The effects of ethanol and barbiturate tolerance have also been examined because, although there is little evidence that barbiturates affect dopamine neurons, the production of tolerance and physical dependence by different types of drugs in the rat could have some common neurochemical features.

MATERIALS AND METHODS

Female Sprague-Dawley rats had the nigrostriatal dopamine neurons on one side lesioned by injection of 40 μg of 6-OHDA hydrobromide in sterile saline containing 1 mg ml^{-1} of ascorbic acid into the left striatum at the coordinates A 8·5 mm; L 2·6 mm; H −1·0 mm (König & Klippel, 1963). After 21 days (+)-amphetamine sulphate injected intraperitoneally produced vigorous ipsilateral circling. The circling rate was recorded using rotameters similar to that described by Ungerstedt (1971). The mean circling rate (turns min^{-1}) of groups of rats was obtained by recording the total turns during 45 min starting 15 min after amphetamine injection.

Lesioned rats were made tolerant to and physically dependent on morphine by implanting subcutaneously at the back of the neck two tablets each containing 75 mg of morphine alkaloid and prepared as described by Gibson & Tingstad (1970). After 4 days implantation, naloxone (2 mg kg^{-1}, i.p.) immediately produced the withdrawal symptoms of morphine. For a permanent withdrawal the tablet residues were removed.

Ethanol was administered chronically by substituting a solution of 10% (v/v) absolute ethanol in

1·5% (w/v) sucrose for drinking water. After one week the concentration of ethanol was increased to 15% and this was maintained for two more weeks, during which the average daily dose was 10 g kg^{-1}.

Tolerance and physical dependence to barbiturate was induced by adding barbitone sodium to the drinking water sweetened with saccharin sodium. A starting dose of 100 mg kg^{-1} of barbitone daily was increased each week by 100 mg kg^{-1} until 400 mg kg^{-1} daily was reached. This dose was maintained for two weeks.

RESULTS

Amphetamine caused dose-related ipsilateral circling in 6-OHDA lesioned rats (Table 1). The effects of chronic drug treatment were investigated using the

Table 1. *Dose dependency of the circling behaviour induced by (+)-amphetamine sulphate in rats with unilateral 6-OHDA striatal lesions.*

Dose of (+)-amphetamine sulphate (mg kg^{-1}, i.p.)	Mean ipsilateral circling rate (turns min^{-1})
0	0·2 ± 0·03
1·0	1·4 ± 0·34
2·5	5·7 ± 0·48
5·0	16·9 ± 1·63
7·5	21·6 ± 0·92

Each result is the mean circling rate recorded between 15 and 60 min after injection in groups of 6 rats.

submaximal dose of 5 mg kg^{-1} of (+)-amphetamine sulphate (equivalent to a dose of 1·8 mg kg^{-1} of (+)-amphetamine). This dose produced a mean circling rate of 18·3 ± 0·4 turns min^{-1} in a group of 12 lesioned rats. Each rat was then implanted with two morphine tablets. Tolerance to the initial catalepsy and respiratory depression developed after two days; after three days the rats were behaving almost normally. The amphetamine circling response was tested after 4 days of morphine treatment. The mean circling rate was then significantly reduced (Fig. 1). Naloxone (2 mg kg^{-1}) was given on day 5. This promptly caused a withdrawal syndrome with teeth chattering episodes and 'wet-dog' shakes. No spontaneous circling was seen during withdrawal. After 60 min when the withdrawal signs had subsided the response to amphetamine was tested again. The circling rate was significantly increased compared with the response obtained before morphine treatment. The increased sensitivity to amphetamine was temporary. It was still evident 14 days after withdrawal but after 21 days the circling rate was back to normal.

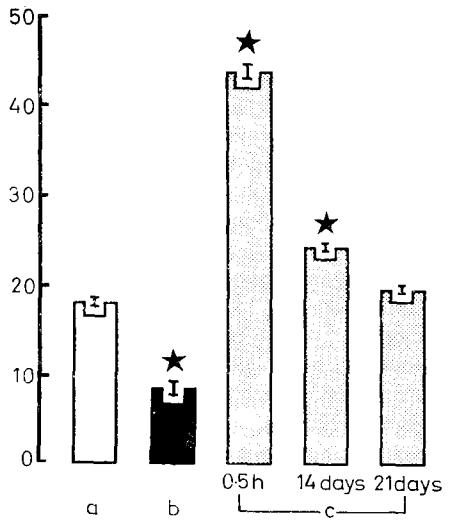

FIG. 1. The mean (+)-amphetamine circling recorded in a group of 12 6-OHDA lesioned rats (a) before chronic morphine; (b) after 4 days of chronic morphine treatment (solid bar) and (c) after morphine withdrawal (dotted bars) for the periods specified. The bars marked * differ significantly from the before morphine result ($P < 0·05$). Ordinate: Mean circling rate (turns min^{-1}).

A further group of 12 lesioned rats were treated chronically with barbitone for 4 weeks during which the daily intake reached 400 mg kg^{-1}. This was almost entirely taken during the night and caused early morning sedation which disappeared later in the day. Circling measurements were made in the afternoons. After four weeks the mean amphetamine circling rate was significantly depressed (Fig. 2). Withdrawal of the barbitone for 48 h produced no obvious physical signs. Noise was avoided since barbiturate withdrawn rats are known to be susceptible to audiogenic seizures (Stevenson & Turnbull, 1968).

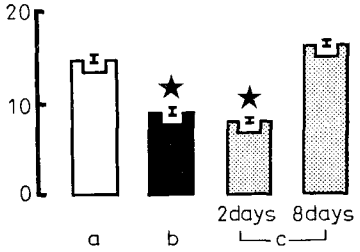

FIG. 2. The mean (+)-amphetamine circling recorded in a group of 12 6-OHDA lesioned rats (a) before chronic barbitone; (b) after 28 days of chronic barbitone treatment and (c) after barbitone withdrawal for the periods specified. The bars marked * differ significantly from the before barbitone result ($P < 0·05$). Ordinate: Mean turns min^{-1}.

The circling response of the 48 h withdrawn rats was also significantly depressed but the amphetamine response returned 8 days after withdrawal.

A single dose of ethanol (5 g kg^{-1}) given by intragastric intubation caused sedation. A group of 6 lesioned rats were tested with amphetamine 30 min after this dose. Their mean circling rate was reduced by 50% from 13·7 ± 0·3 to 6·8 ± 0·3 turns min^{-1}. After 21 days treatment with ethanol, including 14 days when the animals were drinking 10 g kg^{-1} daily, there was no change in the circling response to amphetamine (Fig. 3). Ethanol withdrawal produced no signs of physical dependence although the animals must have become tolerant because no sedation was seen before withdrawal. After 48 h withdrawal the rats were tested again with amphetamine. The mean circling rate was the same as that recorded before chronic ethanol treatment.

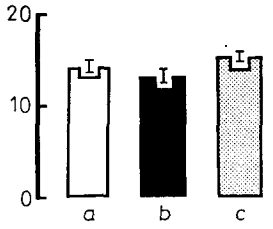

FIG. 3. The mean (+)-amphetamine circling recorded in a group of six 6-OHDA lesioned rats (a) before chronic ethanol; (b) after 21 days of chronic ethanol treatment and (c) after ethanol withdrawal. Ordinate: Mean turns min^{-1}.

DISCUSSION

There is clear evidence that changes in the biochemistry of brain dopamine accompany both acute and chronic administration of morphine. The dopamine receptor blocking drug haloperidol not only potentiates morphine analgesia and enhances tolerance in mice (Eidelberg & Erspamer, 1975) but also blocks some withdrawal signs in both rats and man (Lal, Puri & Karkalas, 1971). It has been suggested that the acute effects of morphine in the rat include a reduction of the amount of dopamine released by striatal nerve terminals (Kuschinsky & Hornykiewicz, 1974; Lal, 1975; Blundell & others, 1976). The present results demonstrate that withdrawal of the morphine-tolerant animal with naloxone results in an increased circling response to amphetamine and support the hypothesis that chronic treatment with morphine initially suppresses dopamine release which is eventually compensated for either by the release of more dopamine or by an increase in the sensitivity of postsynaptic dopamine receptors. The dopamine changes are reversible, the amphetamine response returning to normal several weeks after morphine withdrawal.

The acute effect of barbitone on amphetamine circling was not investigated because it is known that pentobarbitone in a subanaesthetic dose has little effect (Pycock, Tarsy & Marsden, 1975) and that an anaesthetic dose was needed before any changes in brain noradrenaline and dopamine turnover occurred (Corrodi & others, 1966; Persson & Waldeck, 1971). Relatively little is known of the role of brain neurotransmitters in barbiturate dependence. Rats treated with 6-OHDA are more susceptible to barbiturate withdrawal seizures (Morgan, 1976) which suggests a role for catecholamines. The amount of barbitone administered in this study is known to produce physical dependence as well as the more obvious tolerance. Sudden withdrawal of barbiturate from dependent rats precipitates a withdrawal syndrome involving weight loss and a greatly increased susceptibility to seizures, especially audiogenic seizures (Essig, 1966; Stevenson & Turnbull, 1968). The present results show that tolerance to barbitone leads to a reduction in amphetamine-induced circling which is almost certainly not because the animals were sedated since the turning experiments were made when sedation had disappeared and also because the reduced amphetamine circling response was present up to 24 h after withdrawal. Thus the effects of tolerance and withdrawal of a barbiturate on striatal dopamine neurons are different from the effects of morphine.

Some central actions of ethanol might be mediated through brain catecholamines (Carlsson, Engel & Svensson, 1972). While acute doses of ethanol have no effect upon dopamine synthesis in rat striatum (Bustos & Roth, 1976), these authors proposed that ethanol increases the impulse activity in dopaminergic neurons. It is relatively easy to produce tolerance to ethanol in rats and mice but it is much more difficult to induce physical dependence with definite withdrawal signs. Withdrawal signs described include tremors and convulsions (Hunt, 1973) while on occasions it has not been possible, as in this study, to produce evidence of dependence (McQuarrie & Fingl, 1958; Ratcliffe, 1972). The present results show that ethanol withdrawal in the absence of physical signs fails to effect any observable change in striatal dopamine neurons.

It is clear that the three drugs examined each had a different effect on striatal dopamine neurons. Although the evidence that morphine affects dopamine

neurons directly is substantial, nevertheless it is not conclusive. Other neurotransmitters are released in the striatum such as acetylcholine, 5-HT and GABA. It has been suggested that 5-HT neurons from the median raphe are involved in the mediation of circling behaviour (Costall & Naylor, 1974). At present it is not possible to say whether morphine affects dopamine neurons by way of another neurotransmitter.

Acknowledgment

Naloxone was a gift from Endo Laboratories.

REFERENCES

BLUNDELL, C., CROSSMAN, A. R. & SLATER, P. (1976). *Br. J. Pharmac.*, **56**, 456P.
BUSTOS, G. & ROTH, R. H. (1976). *J. Pharm. Pharmac.*, **28**, 580–582.
CARLSSON, A., ENGEL, J. & SVENSSON, T. H. (1972). *Psychopharmacologia*, **26**, 307–313.
CORRODI, H., FUXE, K. & HÖKFELT, T. (1966). *J. Pharm. Pharmac.*, **18**, 556–558.
COSTALL, B. & NAYLOR, R. J. (1974). *Eur. J. Pharmac.*, **29**, 206–222.
COX, B., ARY, M. & LOMAX, P. (1976). *J. Pharmac. exp. Ther.*, **196**, 637–641.
DARDEN, J. & HUNT, W. A. (1975). *Pharmacologist*, **17**, Abs 240.
DURITZ, G. & TRUITT, E. B. (1966). *Biochem. Pharmac.*, **15**, 711–721.
EFRON, D. H. & GESSA, G. L. (1963). *Archs int. Pharmacodyn. Thér.*, **142**, 111–116.
EIDELBERG, E. & ERSPAMER, R. (1975). *J. Pharmac. exp. Ther.*, **192**, 50–57.
ESSIG, C. F. (1966). *Int. J. Neuropharmac.*, **5**, 103–107.
GIBSON, R. D. & TINGSTAD, J. E. (1970). *J. pharm. Sci.*, **59**, 426–427.
HUNT, W. A. (1973). *Neuropharmac.*, **12**, 1097–1102.
HUNT, W. A. & MAJCHROWICZ, E. (1974). *J. Neurochem.*, **23**, 549–552.
KAROUM, F., WYATT, R. J. & MAJCHROWICZ, E. (1976). *Br. J. Pharmac.*, **56**, 403–411.
KÖNIG, J. F. R. & KLIPPEL, R. A. (1963). *The Rat Brain*. Baltimore: Williams & Wilkins.
KUSCHINSKY, K. & HORNYKIEWICZ, O. (1974). *Eur. J. Pharmac.*, **26**, 41–50.
LAL, H. (1975). *Life Sci.*, **17**, 483–496.
LAL, H., PURI, S. K. & KARKALAS, Y. (1971). *Pharmacologist*, **13**, 263.
MCQUARRIE, D. G. & FINGL, E. (1958). *J. Pharmac. exp. Ther.*, **124**, 264–271.
MORGAN, W. W. (1976). *Experientia*, **32**, 489–491.
PERSSON, T. & WALDECK, B. (1971). *J. Pharm. Pharmac.*, **23**, 377–378.
PYCOCK, C., TARSY, D. & MARSDEN, C. D. (1975). *Psychopharmacologia*, **45**, 211–219.
RATCLIFFE, F. (1972). *Archs int. Pharmacodyn. Thér.*, **196**, 146–156.
STEVENSON, I. H. & TURNBULL M. J. (1968). *Biochem. Pharmac.*, **17**, 2297–2305.
UNGERSTEDT, U. (1971). In: 6-*Hydroxydopamine and Catecholamine Neurones*, Editors Malmfors, T. & Thoenen, H., Amsterdam: North-Holland.