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The differential effects of morphine, oxotremorine and antipsychotic drugs on DOPAC concentrations in rat brain

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The effects of morphine and oxotremorine on concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) in the rat striatum and tuberculum olfactorium (TO) have been compared with the effects of the antipsychotic drugs haloperidol, chlorpromazine and clozapine. All the drugs elevated DOPAC concentrations in both brain regions. While the dose-response curves for the antipsychotic drugs were parallel, had steep slopes and similar maxima, the curves for morphine and oxotremorine were irregularly shaped but the curve for morphine in the TO had some similarity to that of the antipsychotic drugs. From these findings, it is concluded that the dose-dependent increase in striatal DOPAC effected by antipsychotic drugs can be used to differentiate them from other drugs known to elevate dopamine metabolites.

Morphine and oxotremorine elevate the dopamine (DA) metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the striatum and mesolimbic areas of the rat brain (Kuschinsky & Hornykiewicz 1972; Nose & Takemoto 1974; Westerink et al 1977). Antipsychotic drugs also increase DA metabolites in the same areas (Wilk et al 1975a; Westerink et al 1977; Stanley & Wilk 1977). Both morphine and antipsychotic drugs enhance pituitary release of prolactin, the regulation of which is thought to be under dopaminergic control (Heinberg et al 1971; Tolis et al 1978). Langer et al (1977) and Meltzer et al (1977) have suggested that a compound's ability to increase circulating prolactin concentrations is a useful means for determining antischizophrenic potency.

In addition to its biochemical effects on dopaminergic systems, morphine also elicits behavioural effects that are similar to those induced by antipsychotic drugs. For example, like chlorpromazine and haloperidol, morphine is an effective inducer of catalepsy and also antagonizes conditioned avoidance responding (Kuschinsky & Hornykiewicz 1972; Stanley & Glick 1976; Worms & Lloyd, in press). Gold et al (1978) have suggested that, because of its antidopaminergic properties, morphine might be an effective antipsychotic.

We have previously found the dose-dependent increase in DA metabolites in rat striatum to be an accurate indicator of antipsychotic potential (Wilk & Stanley 1977). By measuring the accumulation of DOPAC, the principal metabolite of dopamine in the rat brain (Wilk et al 1975b; Westerink & Korf 1976; Karoum et al 1977), we have been able to distinguish clinically active compounds from their inactive analogues (in spite of their activity in a variety of behavioural tests) (Stanley & Wilk 1977). Using this system we have been able to predict the clinical potency of thiethylperazine (Rotrosen et al 1978), a phenothiazine previously regarded as clinically inactive (Matthysse 1973), and the atypical DA antagonist metoclopramide (Stanley & Wilk 1979) which has recently been shown to possess antipsychotic activity (Stanley et al 1979).

We have therefore determined whether the antidopaminergic properties displayed by oxotremorine and morphine could be differentiated from those of known antipsychotic drugs. Accordingly, we have tested both morphine and oxotremorine for their ability to cause a dose-dependent increase in DOPAC concentrations in the striatum and TO and compared this with the effects of haloperidol, chlorpromazine and clozapine.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Perfection Breeders, Douglasville PA), 175–200 g, were housed within the Mt. Sinai animal facility which has a 12-h light/dark cycle (light 0700–1900 h) at 25 °C and 60% humidity.

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Chemicals and Drugs

Pentafluoropropionic anhydride was obtained from Pierce Chemicals, Rockford, Illinois. Pentafluoropropanol was from Peninsula Chemical Research Company, Gainesville, Florida. The reagents were purified by fractional distillation. DOPAC was from the Sigma Chemical Company, St Louis, Missouri. 3,4-Dihydroxyphenylpropionic acid was from the Aldrich Chemical Company, Milwaukee, Wisconsin. JXR (3%) coated on Gas-Chrom Q 100/120 mesh was from the Applied Science Laboratories, Inc., State College, Pennsylvania. Haloperidol (McNeil), chlorpromazine (SKF) and clozapine (Sandoz-Wander, Inc.) were generous gifts. Methylatropine nitrate and oxotremorine were from Sigma. Morphine sulphate was dissolved in 0·9% NaCl (saline). Stock solutions of haloperidol and clozapine were prepared by dissolving both compounds in 0·2 M acetic acid and diluting the solution with saline. All drugs were administered i.p. on a mg kg^{-1} basis. Animals receiving oxotremorine were pretreated with 10 mg kg^{-1} of methylatropine 15 min before receiving oxotremorine.

Tissue preparation

Animals were kept at room temperature until decapitated. Brains were rapidly removed and dissected at room temperature (approx. 3 min). A transverse scalpel cut was made anterior to the hypothalamus at the level of the anterior commissure through the optic chiasm. Left and right striata on either side of the cut were dissected and combined. Left and right tuberculi olfactorium (TO) were dissected from the anterior brain slice and combined. The TO was defined laterally by the lateral olfactory tract, medially by the most medial part of the anterior commissure and dorsally by a plane tangential to the lateral olfactory tract.

Striata and TO from individual rats were homogenized separately in 1 ml of cold 1 M HCl and centrifuged in the cold (4 °C) for 20 min at 15 000 rev min^{-1}. DOPAC was determined in 0·1 ml of supernatant from striatum and 0·25 ml of supernatant from the TO by the method of Watson et al (1974).

RESULTS

As a dose of 1 mg kg^{-1} of oxotremorine and 10 mg kg^{-1} of morphine were found to cause a significant elevation of DOPAC, these doses were used for a time-action study. Animals receiving oxotremorine were killed 15, 30 and 60 min after its administration, while morphine-treated rats were killed after 15, 30, 60, 120 and 240 min.

The results indicated that both drugs had their peak effect on DOPAC concentrations in the striatum and TO at about 1 h (Figs 1a, b; 2a, b). (The response to oxotremorine in the TO did not follow a strict time-related behaviour and further increases in dose or extension of time beyond 1 h were not attempted because of the drug's toxicity), thus the 1 h point was chosen for assessing the dose-response effects of both drugs.

Unlike the antipsychotic drugs, which produce a typical dose-dependent increase in striatal DOPAC, morphine and oxotremorine showed no strict dose-response relationship (Fig. 3). The curve for oxo-

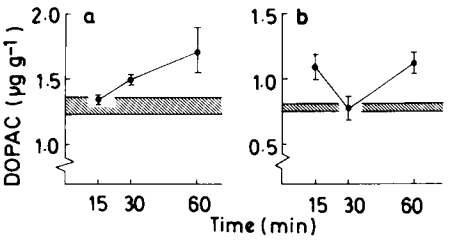

FIG. 1. Time-action curves for the increase in DOPAC in the striatum (a) and TO (b) following i.p. administration of 1 mg kg^{-1} of oxotremorine. Hatched area represents the mean ± s.e.m. of 20 saline-treated rats (striatum 1·17 μg g^{-1} ± 0·04–TO 0·8 μg g^{-1} ± 0·03). All differences are statistically significant ($P < 0·01$) except for those seen at 15 min in striata (a) and 30 min in TO (b).

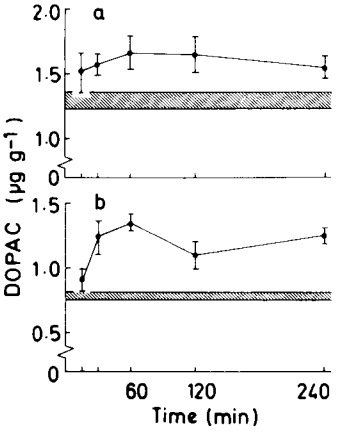

FIG. 2. Time-action curves for the increase in DOPAC in the striatum (a) and TO (b) following i.p. administration of 10 mg kg^{-1} of morphine. Hatched area represents the mean ± s.e.m. of 20 saline-treated rats (striatum 1·17 μg g^{-1} ± 0·04–TO 0·80 μg g^{-1} ± 0·03). Morphine resulted in significant ($P < 0·01$) increases in DOPAC concentrations at all times except the 15 min point in the TO.

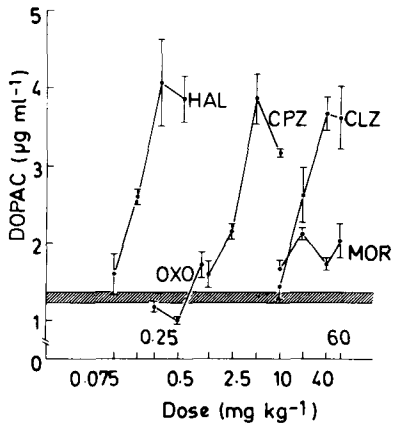

FIG. 3. Dose-response curves for the increase in striatal DOPAC following i.p. administration of haloperidol (HAL), chlorpromazine (CPZ), clozapine (CLZ), oxotremorine (OXO) and morphine (MOR). Each point represents the mean ± s.e.m. of 4 rats. Hatched area represents mean ± s.e.m. of 20 saline-treated rats (1·17 μg g^{-1} ± 0·04).

tremorine in the TO was similar to that seen with the striatum (Figs 3, 4), while the effect of morphine on DOPAC accumulation in the TO more closely resembled the response seen after treatment with antipsychotics (Fig. 4).

DISCUSSION

The results show that the effects of morphine and oxotremorine on DOPAC concentrations in the striatum clearly differ from the effects of the antipsychotic drugs tested. The increase in DA turnover after treatment with drugs like haloperidol, is in agreement with previous findings (Wilk et al 1975a; Cowan et al 1976; Stanley & Wilk 1977). The moderate increase in striatal DOPAC produced by morphine has been observed by DeMontis et al

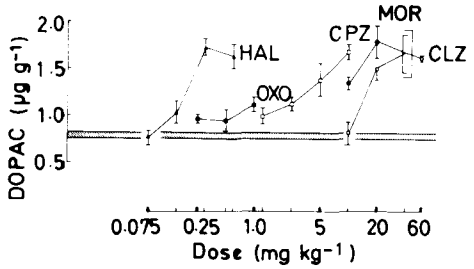

FIG. 4. Dose-response curves for the increase in DOPAC in the TO following i.p. administration of haloperidol (HAL), chlorpromazine (CPZ), clozapine (CLZ), oxotremorine (OXO) and morphine (MOR). Each point represents the mean ± s.e. of 4 rats. Hatched area represents the mean ± s.e. of 20 saline-treated rats (0·80 μg g^{-1} ± 0·03).

(1978) who used a 25 mg kg^{-1} dose. Oxotremorine also produces a moderate elevation in HVA in the rat striatum and limbic system (Bartholini et al 1975) and in the rabbit striatum and limbic system (Andén 1974; Andén & Wachtel 1977). Physostigmine also causes an increase in DA turnover (Andén & Wachtel 1977).

Morphine's weak effect on striatal DOPAC contrasts with its ability to induce profound catalepsy in animals (Kuschinsky & Hornykiewicz 1972), a prototypical indicator of extrapyramidal side effects in man (Hill & Tedeschi 1971). Kuschinsky & Hornykiewicz (1972) have reported that at 10 mg kg^{-1} morphine causes catalepsy in 100% of animals. However, recent studies have indicated that the striatum may not be the primary locus of action of narcotics in inducing catalepsy. Thus bilateral lesions of the striatum attentuated chlorpromazine-induced catalepsy in the rat whereas morphine-induced catalepsy was enhanced by this treatment (Koffer et al 1978). There are other differences between antipsychotics and narcotics, for example, the morphine-induced increase in HVA is antagonized by naloxone, while the chlorpromazine-induced increase in HVA is not (Kuschinsky & Hornykiewicz 1972). Burt et al (1976) have reported that morphine is inactive in competing for [^{3}H]haloperidol binding. Leysen et al (1977) found there was no correlation between IC50 values for binding at opiate and neuroleptic receptors. These findings further suggest that narcotics and antipsychotics act via different mechanisms in the rat brain.

Of interest is the more pronounced effect of morphine on DOPAC concentrations in the TO compared with the striatum. Morphine's effect on DA metabolism in the TO closely resembles the effects seen after typical antipsychotic drugs. It has been suggested that the limbic regions may be the sites mediating the action of antipsychotic drugs (Andén & Stock 1973; Matthysse 1973). Morphine has not so far been shown to have a clinical spectrum of action similar to known antipsychotic compounds.

It could therefore appear that striatal DOPAC concentrations can be used to differentiate antipsychotic drugs from other types of centrally-active compounds known to increase DA metabolites.

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REFERENCES

Andén, N. E. (1974) J. Pharm. Pharmacol. 26: 738–740

Andén, N. E., Stock, G. (1973) Ibid. 25: 346–348

Andén, N. E., Wachtel, H. (1977) Ibid. 29: 435–437

Bartholini, G., Keller, H. H., Pletscher, A. (1975) Ibid. 27: 439–442.

Burt, D. R., Creese, I., Snyder, S. H. (1976) Mol. Pharmacol. 12: 800–812

Cowan, A., Dettmar, P. W., Walter, D. S. (1976) Proc. of B.P.S., July 15–16, Br. J. Pharmacol. 58: 275

DeMontis, G. M., Olianas, M. C., DiLorenzo, C., Tagliamonte, A. (1978) Eur. J. Pharmacol. 47: 121–123

Gold, M. S., Redmond, D. E., Donabedian, R. K., Goodwin, F. K., Extein, I. (1978) Am. J. Psychiatry 135: 1415–1416

Heinberg, D. L., Noel, G. L., Frantz, A. G. (1971) J. Clin. Endocrinol. Metab. 33: 873–876

Hill, R. T., Tedeschi, D. H. (1971) in: Rech, R. H., Moore, K. E. (eds) An Introduction to Psychopharmacology. Raven Press, New York, pp 237–288

Karoum, F., Neff, N. H., Wyatt, R. J. (1977) Eur. J. Pharmacol. 44: 311–313

Koffer, K. B., Berney, S., Hornykiewicz, O. (1978) Ibid. 47: 81–86

Kuschinsky, K., Hornykiewicz, O. (1972) Ibid. 19: 119–122

Langer, G., Sachar, E. J., Gruen, P. H., Halpern, F. S. (1977) Nature (London) 266: 639–640

Leysen, J., Tollenaere, J. P., Koch, M. H. J., Laduron, P. (1977) Eur. J. Pharmacol. 43: 253–267

Matthysse, S. (1973) Fed. Proc. Fed. Am. Soc. Exp. Biol. 32: 200–205

Meltzer, H. Y., Fessler, R. G., Fang, V. S. (1977) Psychopharmacology 54: 183–186

Nose, T., Takemoto, H. (1974) Eur. J. Pharmacol. 25: 51–55

Rotrosen, J., Angrist, B. M., Gershon, S., Aronson, M., Gruen, P., Sachar, E. J., Denning, R. K., Matthysse, S., Stanley, M., Wilk, S. (1978) Arch. Gen. Psychiatry 35: 1112–1118

Stanley, M. E., Glick, S. D. (1976) Neuropharmacology 15: 393–394

Stanley, M., Wilk, S. (1977) Eur. J. Pharmacol. 44: 293–302

Stanley, M., Wilk, S. (1979) Life Sci. 24: 1907–1912

Stanley, M., Lautin, A., Rotrosen, J., Gershon, S. (1979) IRCS Med. Sci. 7: 322

Tolis, G., Dent, R., Guyda, H. (1978) J. Clin. Endocrinol. Metab. 47: 200–203

Watson, E., Travis, B., Wilk, S. (1974) Life Sci. 15: 2167–2178

Westerink, B. H. C., Korf, J. (1976) Eur. J. Pharmacol. 37: 249–255

Westerink, B. H. C., LeJune, B., Korf, J., Van Praag, H. M. (1977) Ibid. 42: 179–190.

Wilk, S., Stanley, M. (1977) Eur. J. Pharmacol. 41: 75–72

Wilk, S., Watson, E., Stanley, M. E. (1975a) J. Pharmacol. Exp. Ther. 195: 265–270

Wilk, S., Watson, E., Travis, B. (1975b) Eur. J. Pharmacol. 30: 238–243

Worms, P., Lloyd, K. G. in: Zbinden, G., Gross, F. (eds) Pharmacological Methods in Toxicology, Pergamon Press, London, in press.