# EFFECT OF METHADONE ON BRAIN DOPAMINE METABOLISM

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PREVIOUS studies from our laboratory (SASAME *et al.*, 1972) have shown that methadone shares with phenothiazine and butyrophenone neuroleptics several pharmacological and biochemical actions. Thus, methadone causes catalepsy and hypothermia in rats, and the latter effect is more pronounced in animals kept in a cold environment. It also blocks apomorphine-induced gnawing, increases the brain HVA level, but not that of 5-HIAA, and stimulates the synthesis of brain DA. Because the cataleptic action of methadone is potentiated by α-methyl-tyrosine (α-MT), and reversed by apomorphine we suggested that this effect is secondary to an inhibition by methadone of dopaminergic receptors: that is the increase in DA synthesis found after methadone administration may be a compensatory feedback response to the blockade of dopaminergic receptors. Indeed a similar theory has been proposed for neuroleptics. This hypothesis was supported by the finding that apomorphine prevented methadone-induced accumulation of HVA levels.

From a point of view of the structure–activity relationship it is possible to recognise in the molecule of methadone the chetone group and the tertiary amine separated by a three carbon chain as in the haloperidol molecule.

On the other hand, KUSCHYINSKY and HORNYKIEWICZ (1972) have suggested that the catalepsy induced by morphine and methadone might originate from an increased intraneuronal breakdown of the newly formed DA with a resultant decrease of the amine at the receptor sites. According to either theory the increase in DA turnover by methadone or morphine would be secondary to the functional deficiency of DA at receptor sites.

However, several considerations suggest that methadone might exhert an amphetamine like effect at the DA nerve terminals in addition to its inhibitory action at DA receptor sites. Thus, the increase in HVA levels and DA synthesis produced by methadone lasts much longer that the cataleptic response the drug. Moreover, the present results show that methadone stimulates DA turnover in mice, in which it does not produce catalepsy but increased motor activity; this effect, as in the case of *d*-amphetamine is blocked by α-MT. Moreover, DL-methadone, similarly to *d*-amphetamine inhibits monoamine-oxidase (MAO) both *in vitro* and *in vivo*.

Finally, the compound appears to meet the steric requirements of a sympathomimetic agent since in its molecule a phenylalkylamino structure is present, which in the flexible molecule of methadone may approximate that of amphetamine.

## MATERIAL AND METHODS

Male Wistar rats of 200–230 g and male albino mice of 20–25 g were used. The animals were kept under the conditions previously described (SASAME *et al.*, 1972).

Brain 5-HT, 5-HIAA, DA and HVA were assayed fluorometrically as previously described (SASAME *et al.*, 1972).

The conversion of labelled tyrosine to labelled DA was measured according to

COSTA and GROPPETTI (1970). Monoamine-oxidase (MAO) activity was measured with the method of KRAJL (1965).

## RESULTS

In agreement with previous results (SASAME *et al.*, 1971, 1972) D,L-methadone significantly increased brain HVA, but did not influence 5-HIAA levels in rats. In addition, Table 1 shows that methadone increased both HVA and 5-HIAA levels in the mouse brain. In this species, the compound did not produce catalepsy but increased motor activity and produced the Straub phenomenon. Moreover, a dose of Haloperidol, which produced only a moderate catalepsy, increased brain HVA both in rats and mice by a much greater extent than did the maximal dose of methadone used.

On the other hand, *d*-amphetamine, which has been shown to increase DA turnover (COSTA and GROPPETTI, 1970) did not increase brain HVA but significantly increased 5-HIAA in the rat brain.

These results prompted us to investigate whether methadone might interfere with the formation of HVA by inhibiting MAO.

Table 2 shows that DL-Methadone concentrations of $10^{-4}$, $10^{-5}$ and $10^{-6}$ M inhibited the MAO activity of rat brain homogenates by 83, 56 and 12 per cent, respectively. Similar degree of inhibition was exerted by *d*-amphetamine. In contrast, haloperidol inhibited MAO activity by about 50 per cent at the highest concentration used.

The finding that methadone is a potent monoamine oxidase inhibitor *in vitro*, raised the question of whether this compound might effectively inhibit the enzyme *in vivo*. We tried to clarify this problem by studying the effect of methadone on the disposition of brain DA and serotonin released by reserpine.

As Table 3 shows, the administration of DL-methadone prevented the accumulation of brain HVA and 5-HIAA produced by reserpine. Interestingly, the combination of methadone and reserpine resulted in a smaller accumulation of these metabolites than did the administration of methadone given alone. It is possible that the drug combination caused an increased concentration of free DA within the nerve endings, which inhibits tyrosine hydroxylase activity.

As these results show, HVA changes do not always reflect parallel and proportional changes in DA turnover. Therefore we studied the effect of DL-methadone on the conversion of ($^3$H) tyrosine to DA in the mouse brain. In Table 4, are reported

TABLE 1. EFFECT OF DL-METHADONE ON THE LEVELS OF BRAIN HVA AND 5-HIAA IN RATS AND MICE.

| Treatment | Dose (mg/kg i.p.) | Rats | | Mice | |
|---|---|---|---|---|---|
| | | HVA (ng/g) | 5-HIAA (μg/g) | HVA (ng/g) | 5-HIAA (μg/g) |
| None | — | 70 ± 8 | 0·54 ± 0·02 | 180 ± 11 | 0·85 ± 0·02 |
| DL-methadone | 10 | 138 ± 15 | 0·52 ± 0·03 | 260 ± 11 | 0·95 ± 0·03 |
| | 20 | 150 ± 11 | 0·65 ± 0·03 | 360 ± 15 | 1·40 ± 0·02 |
| Haloperidol | 5 | 210 ± 15 | | 525 ± 20 | |
| *d*-Amphetamine | 10 | 81 ± 13 | 0·77 ± 0·02 | 180 ± 8 | 0·85 ± 0·04 |

Drugs given 1 hr before death

TABLE 2. EFFECT OF DL-METHADONE, HALOPERIDOL AND *d*-AMPHETAMINE ON THE MAO ACTIVITY (*) OF RAT BRAIN HOMOGENATES.

| Compound | % Inhibition at the molar concentration $10^{-4}$ | $10^{-5}$ | $10^{-6}$ |
|---|---|---|---|
| DL-methadone | 83·4 | 46·6 | 12·2 |
| Haloperidol | 56·3 | 14·2 | 0 |
| *d*-Amphetamine | 100 | 42·8 | 10·6 |

(*) Calculated as % of kynuramine metabolised in 30 min.

TABLE 3. EFFECT OF RESERPINE ON BRAIN HVA AND 5-HIAA IN MICE TREATED WITH DL-METHADONE.

| Treatment | Dose (mg/kg, i.p.) | HVA (ng/g) | 5-HIAA (μg/g) |
|---|---|---|---|
| None | — | 180 ± 11 | 0·85 ± 0·02 |
| Reserpine | 5 | 350 ± 21 | 1·60 ± 0·03 |
| DL-methadone + reserpine | 20 + 5 | 270 ± 12* | 0·90 ± 0·03* |

Reserpine was given 60 min before sacrifice, methadone 15 min prior to reserpine.
* $P < 0{\cdot}001$ in respect to reserpine treated group.

TABLE 4. EFFECT OF DL-METHADONE ON THE CONVERSION OF $^3$H-TYROSINE TO $^3$H-DA IN THE MOUSE BRAIN.

| | S.A. 20 min after the i.p. injection of $H^3$-tyrosine | |
|---|---|---|
| | Tyrosine | DA |
| Controls | 625 ± 12 | 54 ± 3 |
| DL-methadone | 432 ± 21 | 87 ± 11 |

DL-methadone was given 30 min prior to $^3$H-tyrosine

the specific activities (S.A.) of tyrosine and DA 20 min after the intraperitoneal injection of a pulse dose of ($^3$H) tyrosine to control mice and mice treated with DL-methadone. The S.A. of brain tyrosine was 30 per cent lower than normal in mice treated with methadone. On the other hand, the specific activity of brain DA was 40 per cent higher in methadone-treated mice than in control ones.

These results indicate that also in mice, as in rats, DL-methadone accelerates the conversion of $^3$H tyrosine to brain DA. The decrease in brain tyrosine S.A. may be the result of its decreased transport into the brain or its decreased absorption from peritoneal cavity.

## DISCUSSION

The results of the experiments reported and several contrasting effects of methadone might be interpreted by assuming that the drug has two actions on the dopaminergic system: an inhibitory one at DA receptor sites (weaker and more easily surmountable than that of haloperidol), and an amphetamine-like action at DA nerve terminals.

The two actions would produce opposite effects on behaviour and the resultant effect would depend on the dose, the animal species and the experimental condition. As a species, the amphetamine-like effect would prevail in mice and cats, which are usually wildly excited by methadone. The stimulant effect of methadone, similar to

that of *d*-amphetamine, is antagonised by α-methyl tyrosine (α-MT), by haloperidol and chlorpromazine.

In rats, the prevailing effect of methadone during the first hour would be that of a blockade of DA receptors with a resultant catalepsy. As the level of the drug wears off, the amphetamine-like effect would counteract the cataleptic one.

In humans, the usual effect of methadone is one of sedation, but some subjects became very excited by the drug. The difference in response might depend on differences in DA receptors in different animal species or on differences in DA turnover rates.

It is likely that the analgesic effect of these compounds is more related to the amphetamine-like effect than to the blockade of DA receptors. In fact, α-MT antagonises the analgesic effect of morphine, but potentiates catalepsy (see KUSHINSKY and HORNKIEWICZ, 1972). Moreover, haloperidol possesses no analgesic activity. On the other hand, the inhibitory effect of methadone on DA receptors might play a role on its suppressant effect on the withdrawal syndrome in heroin addicts.

## REFERENCES

COSTA E. and GROPPETTI A. (Eds) (1970) In: *Amphetamines and related compounds:* Proc. Mario Negri Inst. for Pharmacological Research, Milan, Italy p. 231, Raven Press, New York.

KRAJL M. (1965) *Biochem. Pharmacol.* **14,** 1683–1685.

KUSCHINSKY K. and HORNYKIEWICZ O. (1972) *Europ. J. Pharmacol.* **19,** 119–122.

SASAME H. A., PEREZ-CRUET J., DICHIARA G., TAGLIAMONTE A., TAGLIAMONTE P., and GESSA G. L. (1972) *J. Neurochem.* **19,** 1953–1957.