HA-966 effects on striatal dopamine metabolism: implications for dopamine compartmentalization

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It has been established that the drugs γ-butyrolactone (GBL; Roth et al 1973) and 1-hydroxy-3-amino-pyrrolidone-2 (HA-966; Hillen & Noach 1971) induce a rapid and temporary increase in the dopamine (DA) content in rat striatum. This DA increase is thought to be caused by interruption of nerve impulse activity in the dopaminergic nigrostriatal pathway, resulting in inhibition of DA release and a concomitant increase in DA synthesis (Walters et al 1973; van Zwieten-Boot & Noach 1975). It is assumed that the DA that is newly synthesized after administration of these drugs is rapidly stored in synaptic vesicles (Walters et al 1973; Roth 1976). This newly synthesized DA can be easily released by electrical stimulation of the nigrostriatal pathway (Murrin & Roth 1976; van Valkenburg & Noach 1978). Thus it could be expected that after GBL or HA-966 the new DA is protected from metabolism by monoamine oxidase (MAO), which should be reflected by rapidly decreasing concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC), the major deaminated DA metabolite. Although Walters & Roth (1972) originally reported a moderate decrease in DOPAC, di Giulio et al (1978) and Broxterman et al (1979) found DOPAC only increases after GBL and HA-966. These findings among others challenged the concept that DOPAC and consequently homovanillic acid (HVA) which is almost entirely formed from DOPAC (Westerink & Korf 1976), are reliable measures of central dopaminergic activity (di Giulio et al 1978).

In the present experiments we tried to gain more insight into the implications of changes in acidic DA metabolite concentrations after nerve impulse flow inhibition by HA-966. We therefore compared the effects of various doses of HA-966 on striatal DA and its acidic metabolites with the effects of this drug on the concentrations of 3 methoxytyramine (3-MT) after MAO inhibition. The latter metabolite is thought (Kehr 1976) to give more reliable information on DA release than DOPAC, since 3-MT is the major methoxylated metabolite formed extraneuronally from DA by the action of catechol-*O*-methyl transferase. The results are discussed in relation to the distribution of DA in different putative pools.

In two series of experiments male Wistar-derived rats from our own breeding department (SPF, 200–250 g) were used. In the first experiment rats were injected (between 9.00 and 10.00 a.m.) with 25, 50 or 100 mg kg^{-1} HA-966 intraperitoneally. Control rats received the vehicle (0·9% w/v NaCl, saline). In the second experiment the rats were injected with pargyline HCl (Sigma 75 mg kg^{-1} i.p.) or with pargyline + HA-966 (100 mg kg^{-1} i.p.) both drugs simultaneously. Controls were given saline injections. The animals were decapitated at different times after the injections. Striata were rapidly dissected on an ice-cooled plate and immersed in liquid N_2 within 90–120 s after killing. They were weighed and homogenized in 1 ml 0·4 M perchloric acid. DA, DOPAC, HVA and 3-MT were measured according to Westerink & Korf (1977).

In Fig. 1 the time effect curves after three doses of HA-966 on striatal DA and DOPAC are depicted. A dose-dependent DA increase was seen, reaching a maximal value of 184% at t = 1 h after the highest dose, followed by a gradual decline until control values were attained at t = 3 h. Also, we observed non dose-dependent increases of DOPAC (and HVA, not shown), the only significant difference between the rising parts of DOPAC curves being at 1 h after 50 mg kg^{-1} from the points at the same time after 25 and 100 mg kg^{-1} ($P < 0.05$). After lower doses the metabolite concentrations returned to basal values more quickly, evidently because less DA had accumulated and thus less was available for metabolism.

Experiment 2 (see Fig. 2) was performed to investigate whether these DOPAC changes reflected intra- and/or extraneuronal DA metabolism. In rats injected with 75 mg kg^{-1} pargyline, a potent and rapidly acting MAO inhibitor (Karoum et al 1977), a moderate rise in striatal DA of about 50% occurred until t = 1 h.

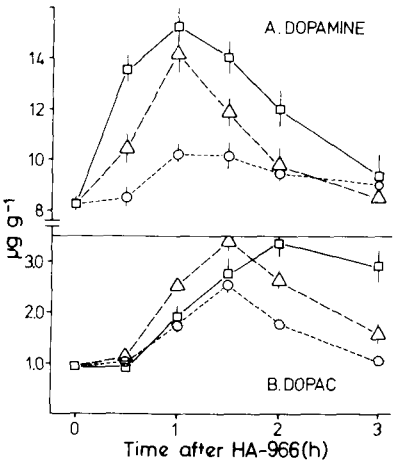

FIG. 1. Effects of HA-966 on striatal dopamine and DOPAC. Rats were injected intraperitoneally with 25 (○---○) 50 (△---△) or 100 (□—□) mg kg^{-1} HA-966. Controls received saline injections. Each value is the mean ± s.e.m. of 5–8 determinations. Ordinate: μg g^{-1}. Abscissa: time after HA-996 (h).

* Correspondence.

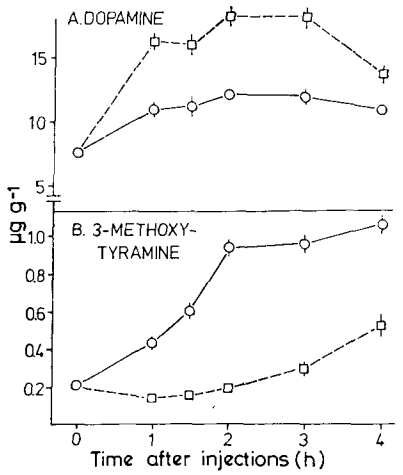

FIG. 2. Effects of HA-966 on striatal dopamine and 3-methoxytyramine after pargyline treatment. Rats were injected with pargyline HCl (75 mg kg^{-1}, i.p. ○—○), or pargyline + HA-966 (100 mg kg^{-1}, i.p. □—□) both drugs simultaneously at zero time. Control animals received saline injections. Each value is the mean ± s.e.m. of 6–9 determinations. Ordinate: $\mu g\ g^{-1}$. Abscissa: time after injections (h).

Thereafter no change was seen during the time measured (4 h). Concomitantly an approximtely linear increase of 3-MT was observed until t = 2 h, when a maximal value was reached.

However, when pargyline and HA-966 were injected simultaneously, a much more marked rise of DA was seen, which was even higher than after HA-966 alone, amounting to about 230% of the control value. The observation that, after combining the two drugs, higher DA concentrations are attained than after either drug alone suggests a high tyrosine hydroxylase activity in spite of impaired DA breakdown. This may be explained by the theory that the enzyme is less sensitive to feedback inhibition when nerve impulse flow is blocked (Morgenroth III et al 1976). 3-MT values after pargyline and HA-966 were slightly decreased (at t = 1 and 1·5 h, $P < 0{\cdot}01$) or not different from saline control values (at t = 2 and 3 h). Since we did not kill the animals by microwave irradiation, it may be assumed that some post-mortem accumulation of 3-MT (Groppetti et al 1977) contributed to our values. The sustained low concentration of 3-MT after HA-966 clearly indicates that the bulk of DA present at the time of HA-966 administration and the DA synthesized thereafter is prevented from methylation and thus, by implication, from release during a period of about 3 h. Four hours after HA-966 the DA concentration sharply decreased and 3-MT showed a marked increase, indicating the resumption of DA release. This time course agrees well with that of experiments in which we used the tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine to estimate the duration of impulse blockade by a single dose of HA-966 (Broxterman et al 1979).

Combining the above data, we can conclude that after HA-966 a dose-dependent increase of striatal DA occurred, owing to increased DA synthesis (van Zweiten-Boot & Noach 1975). Moreover, the surplus of DA has been metabolized within 3 h, while nerve impulse dependent DA release is blocked for 3 h by 100 mg kg^{-1} HA-966 as estimated by tyrosine hydroxylase inhibition according to Andén et al (1971). Since 3-MT (exp. 2) began to rise from about 3 h after HA-966, the large DOPAC rises (exp. 1) must be interpreted as resulting from metabolism independent of DA release. The rates of DOPAC rises were unrelated both to HA-966 doses and amount of DA accumulaton, which makes it unlikely that the total storage capacity of the nerve endings for DA was exceeded. Rather, some rate-limiting factor in the intraneuronal deamination process determined the DOPAC rises. The fact that even much higher DOPAC levels may occur in striatum (e.g. approximately ten times the control value after amfonelic acid + haloperidol; Shore 1976) suggests that MAO activity can be very high and is probably not rate-limiting in DOPAC formation in the present experiment. However, relatively little is known about the exact localization of MAO (Agid et al 1973). We think that a more likely rate-limiting factor may be a transport process from the pool where the newly formed DA is accumulated to the MAO-sensitive cytoplasmic sites.

On the basis of these considerations we suggest the existence of at least three DA pools, which may behave differently. The model we propose (Fig. 3) may be regarded as a modification of the one of Dalhström (1973), who suggested the existence of 'young' and 'old' noradrenaline storage granules, based on i.a. electron microscopical data.

The data presented here favour the existence of two differently behaving MAO-resistant DA pools, in addition to the MAO-sensitive, cytoplasmic pool (pool I). Pool II has the following characteristics: i) it is

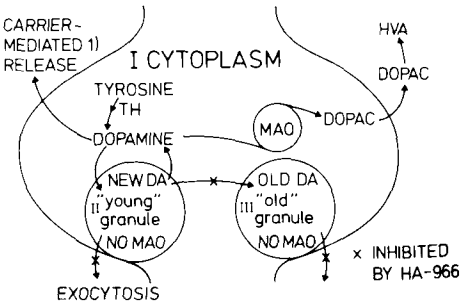

FIG. 3. A hypothetical model for the relation between putative dopamine pools in striatal dopaminergic nerve endings. The model is based on the one of Dahlström (1973) and the difference in behaviour of old and new dopamine after HA-966 administration. See text for further details. (1) See Raiteri et al (1979) J. Pharmacol. Exp. Ther. 208: 195–202.

accessible to newly synthesized DA, but no MAO is present, since DA can accumulate in it. ii) This pool has a fast but rate-limited exchange of DA with pool I, since newly synthesized DA that has accumulated for some time can be metabolized independent of nerve impulses (see the above argument). DA pool II may represent the so-called easy releasable pool (Glowinski 1973). Since the newly formed DA after GBL or HA-966 is readily releasable, e.g. by electrical stimulation (Murrin & Roth 1976; van Valkenburg & Noach 1978), pool II could be granular by nature. However, it clearly differs from pool III of our model consisting of older DA, since from the latter there is no intraneuronal release, followed by deamination, judging from the rapid DOPAC decline after HA-966 + α-methyl-*p*-tyrosine (Broxterman et al 1979). Therefore it seems that pool III is relatively inert and is either not in equilibrium with, or reaches equilibrium only slowly with, DA in the other pools, at least under circumstances of nerve impulse flow inhibition by HA-966. Other authors have provided data showing that also under circumstances of increased impulse flow this pool is not easy to mobilize (Shore & Dorris 1975; Moleman et al 1978).

After HA-966 administration there is probably no transfer of DA to the stable granular pool, since the surplus of DA seems to have been metabolized almost completely at t = 3 h. It may only be speculated that somehow transfer from pool II to pool III (Fig. 3) is impaired after nerve impulse flow inhibition by HA-966. This distinguishes HA-966 (and probably GBL) action from a lesion of the nigrostriatal pathway, since after such a lesion DA values remain elevated for at least 24 h (Andén et al 1972). It is conceivable that under physiological conditions, the transfer of DA from pool II to III in fact is an ageing process of the granules, which would be inhibited by HA-966. Preliminary results in our laboratory showed that DOPAC concentrations are also increased after intrastriatal HA-966 application, indicating that this effect is not directly related to the blocking of nerve activity at the level of the substantia nigra.

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REFERENCES

Agid, Y., Javoy, F., Youdim, M. B. H. (1973) Br. J. Pharmacol. 48: 175–178

Andén, N.-E., Corrodi, H., Fuxe, K., Ungerstedt, U. (1971) Eur. J. Pharmacol. 15: 193–199

Andén, N.-E., Bédard, P., Fuxe, K., Ungerstedt, U. (1972) Experientia 28: 300–301

Broxterman, H. J., Noach, E. L. van Valkenburg, C. F. M. (1979) Eur. J. Pharmacol. in press

Dahlström, A. (1973) Brain Res. 62: 441–460

di Giulio, A. M., Groppetti, A., Cattabeni, F., Galli, C. L., Maggi, A., Algeri, S., Ponzio, F. (1978) Eur. J. Pharmacol. 52: 201–207

Glowinski, J. (1973) Brain Res. 62: 489–593

Groppetti, A., Algeri, S., Cattabeni, F., Di Giulio, A. M., Galli, C. L., Ponzio, F., Spano, P. F. (1977) J. Neurochem. 28: 193–197

Hillen, F. C., Noach, E. L. (1971) Eur. J. Pharmacol. 16: 222–224

Karoum, F., Neff, N. H., Wyatt, R. J. (1977) Ibid. 44: 311–318

Kehr, W. (1976) Naunyn-Schmiedeberg's Arch. Pharmacol. 293: 209–215

Moleman, P., Bruinvels, J., van Valkenburg, C. F. M. (1978) Life Sci. 23: 611–616

Morgenroth, V. H., III, Walters, J. R., Roth, R. H. (1976) Biochem. Pharmacol. 25: 655–661

Murrin, L. C., Roth, R. H. (1976) Naunyn-Schmiedeberg's Arch. Pharmacol. 295: 15–20

Roth, R. H. (1976) in: Hornykiewicz, O. (ed), Pharmacol. Ther. B. 2. Pergamon Press: Oxford, pp 71–88

Roth, R. H., Walters, J. R., Aghajanian, G. K. (1973) in: Usdin, E., Snyder, S. H. (eds) Frontiers in catecholamine research, Pergamon Press, Inc., New York, pp 567–574

Shore P. A. (1976) J. Pharm. Pharmacol. 28: 855–857

Shore, P. A., Dorris, R. L. (1975) Eur. J. Pharmacol. 14: 315–318

van Valkenburg, C. F. M., Noach, E. L. (1978) Eur. J. Pharmacol. 48: 171–177

van Zwieten-Boot, B. J., Noach, E. L. (1975) Ibid. 33: 247–254

Walters, J. R., Roth, R. H. (1972) Biochem. Pharmacol. 21: 2111–2121

Walters, J. R., Roth, R. H., Aghajanian, G. K. (1973) J. Pharmacol. Exp. Ther. 186: 630–639

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

Westerink, B. H. C., Korf, J. (1977) J. Neurochem. 29: 697–706