Haloperidol inhibits the disappearance of acidic dopamine metabolites from rat striatum

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Administration of neuroleptic drugs to laboratory animals results in increased dopamine turnover. This has been shown by use of diverse techniques, like conversion of radioactive tyrosine to dopamine (Nybäck & Sedvall, 1969; Zivkovic, Guidotti & others, 1975), dopamine loss after synthesis inhibition with α-methyl-*p*-tyrosine (α-MT) (Andén, Corrodi & others, 1971; Waldmeier & Maître, 1976) and accumulation of the acidic dopamine metabolites homovanillic acid (HVA) and 3,4-dihydroxyphenyl acetic acid (DOPAC) (Andén, Roos & Werdinius, 1964; Westerink & Korf, 1976b). The latter method has also been used extensively to compare quantatively the effects of different neuroleptics in striatal and mesolimbic brain structures (Stawarz, Hill & others, 1975; Wiesel & Sedvall, 1975; Wilk, Watson & Stanley, 1975; Waldmeier & Maître, 1976; Westerink & Korf, 1976b). A basic assumption in such studies is, that alterations in metabolite concentrations reflect alterations in functional activity of dopaminergic neurons. In the maintainance of normal metabolite concentrations, however, at least two processes are rate-limiting, i.e. the formation of the metabolites and their removal from the brain. The fact that considerable amounts of acidic metabolites are present in normal brain tissue may indicate that the removal from the brain is indeed a rate-limiting process. It seems reasonable to assume that the rate of formation of acidic metabolites is related to the activity of dopaminergic neurons. The concentrations of these metabolites, however, are not only determined by the rate of formation, but also by the rate of transport from the brain. Drug-induced alterations of metabolite concentrations, therefore, cannot be taken as a reflection of the activity of dopaminergic neurons, when the transport of metabolites is also affected by the same drug.

To test whether the increase in the acidic metabolites HVA and DOPAC by haloperidol (Andén, Roos & Werdinius, 1964; Westerink & Korf, 1976b) was at least in part the result of an inhibition of the transport of these metabolites, we inhibited the formation of acidic metabolites with pargyline after which the effect of haloperidol on the metabolite concentrations was studied. Since the formation of acidic metabolites will be inhibited by pargyline, an alteration in metabolite concentrations by haloperidol can only be ascribed to an alteration in the rate of transport (or conjugation). Similarly the effect of the dopamine agonist apomorphine was studied.

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Male Wistar rats, 105–135 g, were used. Pargyline HCl (75 mg kg^{-1}) was injected intraperitoneally, followed within 30 s by injection of haloperidol (1 mg kg^{-1}) or apomorphine (0·5 mg kg^{-1}) into the other side of the peritoneal cavity. Animals were decapitated 15 or 30 min later. Three rats were treated with saline, haloperidol, or apomorphine, respectively, within 8 min and matched for statistical analysis to avoid influences of diurnal fluctuations in dopamine metabolism. The experiments were performed between 9 and 11 a.m. HVA and DOPAC were extracted from single striata, isolated and assayed according to Westerink & Korf (1976b). The values obtained from the two striata of one rat were averaged.

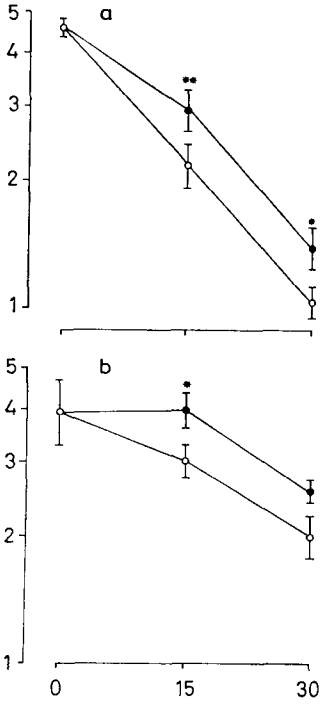

FIG. 1. Effect of haloperidol on the disappearance of the acidic dopamine metabolites DOPAC (a) and HVA (b) from rat striatum after pargyline treatment. Rats were injected intraperitoneally with pargylien HCl (75 mg kg^{-1}) and haloperidol (●——●; 1 mg kg^{-1}) or saline (○——○) at t = 0 min. Number of rats per group: 4–6. See text for details. *$P<0.025$. **$P<0.001$. Paired Student's *t*-test. Ordinate: n mol g^{-1}. Abscissa: Time (min).

Administration of pargyline induced a rapid decrease in DOPAC concentrations (Fig. 1a), while the loss of HVA was less pronounced (Fig. 1b). Fig. 1 shows that haloperidol inhibits the decline of both HVA and DOPAC for 15 min. Values for apomorphine-treated rats were not significantly different from saline (0·9% w/v NaCl) treated rats (not shown). The initial rate of metabolite disappearance was calculated from the concentrations observed at 0 and 15 min after treatment. The values obtained for both DOPAC and HVA were added to obtain a measure of disappearance of acidic metabolites. While in saline-treated rats 17·9 nmol g^{-1} h^{-1} of metabolites disappeared, this value amounted to only 7·7 nmol g^{-1} h^{-1} in haloperidol-treated rats. Thus, haloperidol injection resulted in a 10·2 nmol g^{-1} h^{-1} decrease in the initial rate of disappearance of acidic metabolites, which is a decrease of more than 50%.

Differences between different brain regions in the increase of DOPAC and HVA concentrations by various drugs have repeatedly been reported (Stawarz & others, 1975; Wiesel & Sedvall, 1975; Wilk & others, 1975; Waldmeier & Maître, 1976; Westerink & Korf, 1976b). Attempts to relate these effects to antipsychotic properties or to the liability to induce extrapyramidal side effects did not produce unequivocal results. While some authors observed such relations (Andén & Stock, 1973; Wilk & others, 1975; Bartholini, 1976), this was not confirmed by others (Wiesel & Sedvall, 1975; Waldmeier & Maître, 1976; Westerink & Korf, 1976b). Interaction with the transport of acidic dopamine metabolites could explain some of these discrepancies. Several authors, for example, observed a more pronounced increase in DOPAC and HVA concentrations in the striatum, when compared with limbic structures, for all neuroleptics they studied (Wiesel & Sedvall, 1975; Wilk & others, 1975; Waldmeier & Maître, 1976, Scatton, Bischoff & others, 1977). A more pronounced inhibition of metabolite transport in the striatum *vs* mesolimbic structures is one possible explanation of these observations. Similarly, differences in the potency of various neuroleptics to increase the concentration of acidic metabolites (Wiesel & Sedvall, 1975; Waldmeier & Maître, 1976; Westerink & Korf, 1976b) may be related to differences in potency to inhibit the transport of these metabolites.

The validity of the conclusion that haloperidol inhibits the disappearance of acidic dopamine metabolites depends on the assumption that pargyline effectively blocked the oxidation of dopamine to DOPAC. The exponential decline of DOPAC concentrations after administration of 75 mg kg^{-1} pargyline (i.p.) (Fig. 1a, Karoum, Neff & Wyatt, 1977) would seem to indicate that the inhibition of DOPAC formation is rapid in onset. It also appears that the oxidation of dopamine to DOPAC is blocked completely by pargyline. The outflux of acidic metabolites (DOPAC + HVA) was calculated to amount to 17·9 nmol g^{-1} h^{-1} after MAO-inhibition by pargyline (*vide supra*). The outflux of acidic metabolites has also been calculated after inhibition of dopamine biosynthesis with α-MT. The sum of dopamine + DOPAC + HVA decline was calculated to amount to 19 nmol g^{-1} h^{-1} (unpublished results). Moreover, it has been shown that α-MT inhibits dopamine biosynthesis completely during the period of observation in this experiment (Moleman & Bruinvels, 1976). The close agreement between the two methods (17·9 *vs* 19 nmol g^{-1} h^{-1}) suggests a complete or almost complete inhibition of MAO by pargyline. It does not appear very probable, therefore, that the inhibition of metabolite loss of 10·2 nmol g^{-1} h^{-1} induced by haloperidol can be explained by incomplete inhibition of the oxidation of dopamine to DOPAC. An effect of haloperidol on the conversion of DOPAC to HVA is not precluded in the present experiments. However, such an effect could not explain the present results, since the disappearance of both DOPAC and HVA were inhibited (Fig. 1).

Our results may indicate, that the effect of haloperidol on metabolite transport only lasts for about 15 min. However, we would hesitate to draw such a conclusion, since not much is known about the kinetics of the transport of acidic dopamine metabolites. After pargyline treatment the concentrations drop sharply, even when haloperidol is administered. Under these conditions the effect of haloperidol would be expected to subside quickly, if haloperidol would mainly decrease the maximal velocity of metabolite transport. However, the processes by which HVA and DOPAC disappear from brain tissue are not yet fully characterized (Westerink & Korf, 1976a; Karoum & others, 1977).

In conclusion, our results show that haloperidol strongly inhibits the transport or disappearance of the acidic dopamine metabolites HVA and DOPAC from the rat striatum. To fully appreciate the implications of this observation, more has to be known about the kinetics of transport of acidic metabolites and about the relative importance of metabolite formation and metabolite transport/conjugation. Although it should be stressed that interference with the process of conjugation of these metabolites (Gordon, Markey & others, 1976) could be involved in the observed effect of haloperidol, this would not change the implications of the present study. It is suggested that some effects of drugs on concentrations of dopamine metabolites could be explained by inhibition of metabolite transport.

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Comparison of naloxone-induced platform vertical jumping in the assessment of physical dependence in morphine-dependent mice

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The use of subcutaneous implants of morphine pellets to produce morphine-dependent mice and the observance of withdrawal jumping upon administration of nalorphine was first described by Maggiolo & Huidobro (1961). Way, Loh & Shen (1969) used a modified morphine pellet to produce physical dependence in mice and quantified the degree of dependence by assessing the amount of naloxone required to precipitate jumping of the animals from a round platform. Since then many investigators have adopted the antagonist-induced jumping test as a measure of dependence in mice. However, several investigators, including us, have adopted the more convenient observance of naloxone-induced vertical jumping of mice in clear, plastic chambers (Marshall & Grahame-Smith, 1971; Saelens, Granat & Sawyer, 1971; Weissman, 1973; Takemori, Stesin & Tulunay, 1974a; Takemori, Ward & others, 1974b; Eidelberg, Erspamer & others, 1975; Smits, 1975; Cowan, 1976) instead of observing mice jump off a round platform. It has been suggested that perhaps the two types of jumping differ and results obtained by assessment of the two types of jumping may not be similar (E. L. Way, personal communication). To the present authors, this suggestion seemed unlikely since the vertical jumping test has been used satisfactorily to quantify physical dependence (Marshall & Grahame-Smith, 1971; Smits, 1975), to reliably predict the dependence capacity of narcotic drugs (Saelens & others, 1971; Takemori & others, 1974a, b) and to estimate the antagonistic potentencies of narcotic antagonists (Cowan, 1976). Also, both vertical jumping and jumping from a table by nalorphine-treated, morphine-dependent mice was described in the original report of Maggiolo & Huidobro (1961). Nevertheless, there has not been a systematic study comparing the results obtained by observance of both platform and vertical jumping in morphine-dependent mice. Therefore in an agreement with Dr E. Leong Way, we undertook this comparison.

Male Swiss-Webster mice, 25–30 g, were housed for at least one day in the experimental environment before experimentation and each mouse was used only once. The animals were made physically dependent on morphine by a procedure described earlier (Takemori & others, 1974a). In short, the animals were pretreaated with 50 mg kg^{-1} morphine sulphate three times daily for two days and 100 mg kg^{-1} three times on the third day before subcutaneous implantation of morphine pellets which contained 50 mg of morphine free base. This procedure not only decreased the mortality rate but assured us highly tolerant and dependent animals (Takemori & others, 1974a; Tulunay & Takemori, 1974). Physical dependence was quantified 72 h after the implantation of morphine pellets without the removal of the pellets.

Naloxone-induced platform jumping of morphine-

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