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Enhanced effect of haloperidol and apomorphine after hypophysectomy: pharmacokinetic considerations

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The behavioural effects of both apomorphine (stereotypies) and haloperidol (catalepsy and reversal of stereotypies) were significantly enhanced in hypophysectomized rats compared with sham-operated rats. Both the efficacy and duration of action were increased. Hypophysectomized animals had significantly greater brain haloperidol concentrations than did sham-operated animals. The data suggest that changes in brain drug concentrations following hypophysectomy at least partially explain the behavioural alterations seen in this test situation.

Recently, there has been an increasing interest in the possible hormonal and neuroendocrine control of brain dopamine systems and the actions of dopamine agonists and antagonists notably with regard to dopamine neurons of the median eminence and dopamine-related events at the level of the striatum and nucleus accumbens (cf. Van Loon et al 1977; Gudelsky & Moore 1977; Euvrard et al 1980; Hruska et al 1980; Jenner et al 1981). These sometimes contradictory neurochemical data indicate that hypophysectomy, oestrogens and prolactin all influence dopamine neuron activity (either directly or indirectly). In addition, some limited data are available on the possible behavioural consequences of such interactions. Thus, hypophysectomy increases amphetamine stereotypies (Borison & Diamond 1979), and in ovariectomized rats hypophysectomy potentiates the stereotypic effect of apomorphine (Perry et al 1981). However, in rats, Jenner et al (1981) reported that apomorphine stereotypies were not altered by hypophysectomy in either saline-treated rats or rats withdrawn from chronic haloperidol. In ovariectomized rats, oestradiol benzoate was reported to enhance spiroperidol catalepsy, and also the levels of [^{3}H]spiroperidol in the brain (Chiodo et al 1979).

It would appear from these diverse reports that neuroendocrine functions influence dopamine mediated events. However, another explanation not associated with dopamine neuron function could at least partially intervene. Thus, the neuroendocrine peptides released by the pituitary (e.g. growth hormone, ACTH, prolactin) exert marked permissive effects on physiological activity in general and thyroid and hepatic function in particular. This suggested that at least part of the modification of the effects of dopamine agonists or antagonists following hypophysectomy, or other massive alterations in neuroendocrine status, could be at least partially explained by altered hepatic metabolism of the compounds involved. This would result in drug levels in the brain which are different in hypophysectomized versus non-hypophysectomized rats injected with the same dose (on a mg kg^{-1} basis) of drug.

In order to investigate this question, the following experiments were performed in hypophysectomized and sham-operated rats: (1) an analysis of the effects of apomorphine (stereotypies) and haloperidol (antistereotypic, cataleptogenic); (2) an analysis of the pharmacokinetics of haloperidol in the brain, in parallel to the induction of catalepsy.

MATERIALS AND METHODS

Animals

Male albino Sprague-Dawley rats with a preoperative weight of 140–160 g were used. One to two weeks after hypophysectomy or sham operation (operations were performed at Iffa-Credo, Les Oncins) when the hypophysectomized rats weighed 150–175 g and the sham operated rats 170–200 g. A similar failure to gain weight was recently observed by Jenner et al (1981).

Behavioural experiments

Stereotypies were measured after the subcutaneous (s.c.) administration of apomorphine using a 5-point rating scale (Worms & Scatton 1977; Worms & Lloyd 1979). The scores were noted every 10 min for 1 h post injection and then expressed as an accumulated total score for each animal.

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Catalepsy was estimated by means of the 4-cork test (Worms & Lloyd 1979), using two different rating scales. For the time course of action of haloperidol in the same group of rats, the time maintained in the cataleptic position (maximum 120 s) was noted 30, 60, 90, 120 and 240 min after the intraperitoneal (i.p.) administration of haloperidol and the scores cumulated. For the experiment in which catalepsy and haloperidol levels were measured in the brain of the same animals, an all-or-none criterion (10 s cut-off) was used, and the percent of animals cataleptic was noted. This allowed all animals to be treated in exactly the same manner. In these animals the catalepsy was measured immediately before death (30, 60, 120 and 240 min post haloperidol).

Pharmacokinetic analysis

For the determination of haloperidol levels in the brains of the sham and hypophysectomized rats, the animals were decapitated immediately after the catalepsy measurements, the brains removed and immediately frozen (−80 °C). On the day of the haloperidol estimation, the frozen brains were weighed and then placed in glass tubes with enough 0·05 M hydrochloric acid to reach a total (brain + acid) weight of 8 g.

The brains were then homogenized (Polytron, 30 s). Increasing amounts (1–7 ml) of homogenate, in relation to the time elapsed after haloperidol administration, were transferred to glass-test tubes, and the volume adjusted to 10 ml with distilled water. After the addition of 2·0 ml of M sodium hydroxide and 20 ml of distilled ether, the extraction and estimation of haloperidol were performed as described by Bianchetti & Morselli (1978) for the determination of haloperidol in plasma.

Statistics

For statistical analysis, the Mann-Whitney U test (stereotypies and catalepsy) and the Student's *t*-test were used.

RESULTS

As shown in Fig. 1, at either dose of apomorphine used (0·25 mg kg $^{-1}$, s.c. Fig. 1A or 0·50 mg kg^{-1}, s.c. Fig. 1B), the stereotypies observed were more severe and of a longer duration in hypophysectomized than in sham-operated rats ($P < 0·001$ for either dose) over the times analysed. However, the stereotypic effect of apomorphine in hypophysectomized rats was more sensitive to antagonism by haloperidol than it was in sham-operated rats (Fig. 2).

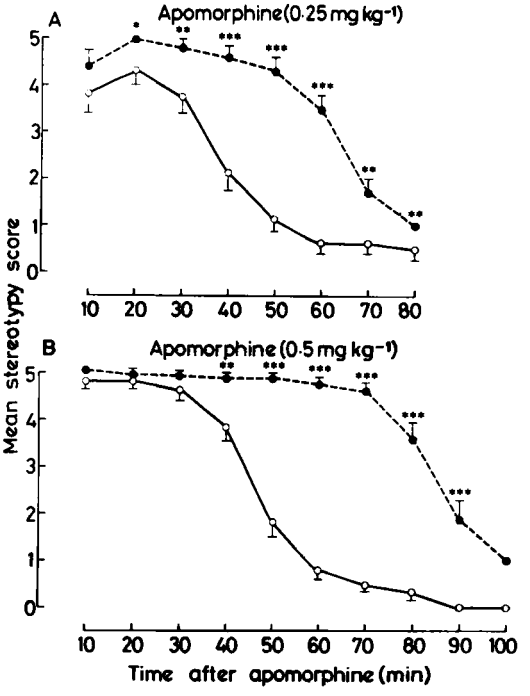

FIG. 1. Time course and severity of apomorphine-induced stereotypies in hypophysectomized (broken line) and sham-operated (solid line) rats (n = 16 rats per group). Values expressed as mean ± s.e.m. * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$ versus sham-operated group.

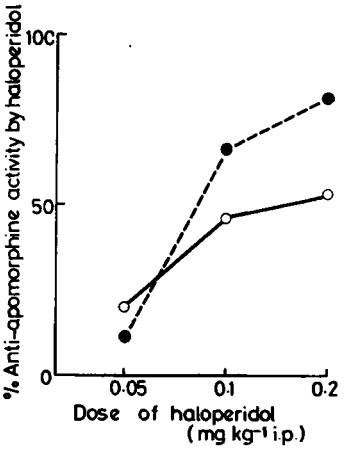

FIG. 2. Anti-apomorphine activity of haloperidol in hypophysectomized and sham-operated rats. Values are expressed as percentage of antagonism. *** $P < 0·001$ versus sham-operated rats.

In view of these latter results, the cataleptic effect of haloperidol was examined. In hypophysectomized animals (Fig. 3), the dose-response curve of haloperidol was shifted considerably to the left indicating an enhanced effect of haloperidol. In terms of the percent of animals responding, a dose of haloperidol (0·1 mg kg^{-1} i.p.), inactive in the shams, produced catalepsy in 33% of the hypophysectomized rats. Similarly at 0·3 mg kg^{-1} only 26% of the sham-operated animals were cataleptic whereas 96% of the hypophysectomized rats demonstrated a clear-cut catalepsy. Even at 1·0 mg kg^{-1} i.p., where most rats were cataleptic (70% of the shams, 98% of the hypophysectomized) the catalepsy induced was of a much longer duration in the hypophysectomized than in sham-operated rats.

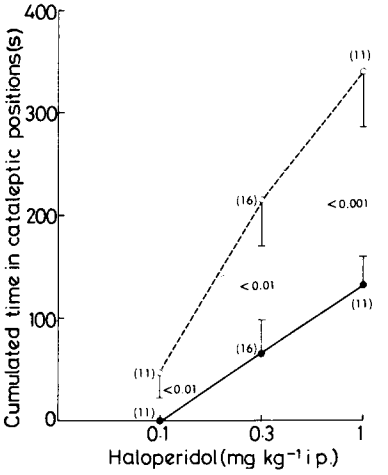

FIG. 3. Haloperidol-induced catalepsy in hypophysectomized (broken line) and sham-operated (solid line) rats. Values are expressed as means ± s.e.m. Number of rats within parentheses.

These experiments were repeated with a modified protocol, for which the animals were decapitated immediately after each catalepsy estimation and the brains removed for later determinations of haloperidol levels (see methods). As shown in Fig. 4, qualitatively the same pharmacological results were obtained as in the previous experiments. Thus, at 0·2 mg kg^{-1} i.p. of haloperidol (Fig. 4A) the hypophysectomized rats were more cataleptic than the sham-operated animals at 1 and 2 h after haloperidol administration. At 30 min neither group exhibited catalepsy and at 4 h both groups exhibited similar, mild catalepsy. In agreement with the results shown in Fig. 3, at the 1, 2 and 4 h times the

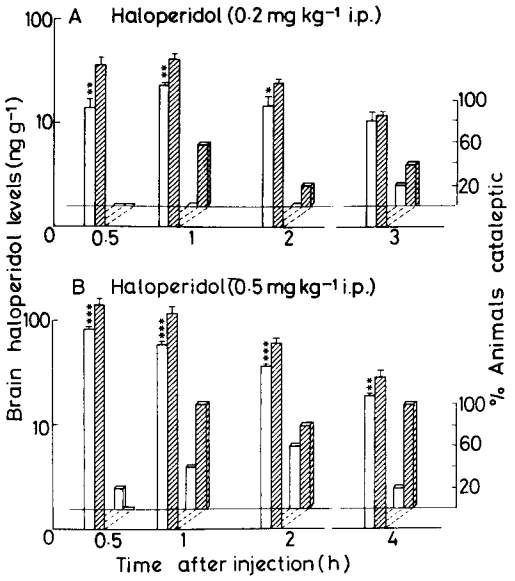

FIG. 4. Brain haloperidol levels and catalepsy induction different time intervals after haloperidol 0·2 mg kg^{-1}, i.p. (A) or 0·5 mg kg^{-1} i.p. (B). Values are expressed as means ± s.e.m. (haloperidol levels) or as percentages (catalepsy). * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$ versus sham-operated rats.

percentage of rats cataleptic tended to be greater in the hypophysectomized group than in the sham-operated group although due to the small number of animals used (n = 5) this was not statistically significant. At three times (0·5, 1·0 and 2·0 h) the haloperidol levels (note the logarithmic scale) were significantly greater (n = 5, $P < 0·01$ for 0 5 and 1·0 h; $P < 0·05$ for 2 h) in the hypophysectomized than in the sham-operated animals.

At a higher dose of haloperidol (0·5 mg kg^{-1} i.p., Fig. 4B) the hypophysectomized animals were again more cataleptic than the shams at 1, 2 and 4 h. In agreement with Fig. 3, at these times the percent of animals cataleptic was greater in the hypophysectomized than in the sham-operated groups. This was statistically significant at 2 and 4 h ($P < 0·05$). Haloperidol levels were also significantly greater (142 to 200% of controls) in the hypophysectomized than in the sham-operated rats ($P < 0·01$ at 0·5, 1·0 and 2·0 h after dosing).

For both doses of haloperidol, the peak brain concentrations were obtained 0·5–1 h after the i.p. administration of the drug. The ratio of the areas under the curves (AUCs) (hypophysectomized/sham) was 1·7 for the 0·2 mg kg^{-1} group and 1·8 for the 0·5 mg kg^{-1} group. The apparent half-life of haloperidol under the different conditions was calcu-

lated, however the values can be considered only as approximate due to the limited number of time points (n = 3 over 1–4 h) available. The values obtained were 3·0 and 2·1 h at 0·2 and 0·5 mg kg^{-1} for the hypophysectomized group, and 2·9 and 1·5 h for the sham-operated rats.

DISCUSSION

These results show that hypophysectomy in the male rat produces an animal which is more sensitive to the effects of both a dopamine agonist (apomorphine) and antagonist (haloperidol). This is in agreement with: (i) the preliminary report by Borison & Diamond (1979) of an increased stereotypic effect of amphetamine and an increased anti-stereotypic effect of haloperidol in hypophysectomized rats; and (ii) the report of Perry et al (1981) on potentiation of apomorphine stereotypies in hypophysectomized, ovariectomized, rats. However, it is not in agreement with the recent observations of Jenner et al (1981) that hypophysectomy does not alter the stereotypic response to apomorphine; the reason for this discrepancy is not readily apparent as a similar dose of apomorphine (0·5 mg kg^{-1} s.c.) was used in both experiments.

Our interpretation of the mechanism of these enhanced effects of the dopamine-related drugs differs from those of the above authors. Thus for the enhanced cataleptogenic effects of haloperidol, part of the potentiation (at least t = 1 and 2 h) is due to increased brain levels of haloperidol, in effect being equivalent to a greater dose of haloperidol being administered. This explanation however does not appear to account for the enhanced effect of haloperidol at longer times (at t = 4 h) when the haloperidol levels had fallen markedly (Fig. 4). The increased levels of haloperidol and the trend towards an increased half-life in the brain could explain the observation of Gudelsky & Moore (1977) that, at much higher doses, haloperidol maintained its effect on striatal dopamine turnover for at least 16 h in hypophysectomized rats whereas in unoperated animals haloperidol was no longer active at this time. Those authors considered (without any supportive evidence) an altered metabolism of haloperidol as one possible explanation.

It is likely that a similar phenomenon (increased drug concentrations) is at least a partial explanation for the enhanced effect of apomorphine. Thus, as (i) the major route of metabolism of apomorphine is via catechol-*O*-methyl transferase (COMT—Mackenzie & White 1973; Missala & Sourkes 1973), (ii) inhibition of COMT activity enhances the stereotypic effects of apomorphine (Symes et al 1975), and (iii) the major source of COMT in the body is the liver (Axelrod et al 1959), in the state of liver atrophy and dysfunction such as exists after hypophysectomy, it could be expected that apomorphine levels will be greatly increased in hypophysectomized compared with sham-operated animals.

This interpretation is not an attempt to refute the hypothesis that the hormonal and neuroendocrine status influence dopamine neuron function and dopamine-mediated events in the brain, as there is interesting, but contradictory, evidence to support this hypothesis (Gudelsky & Moore 1977; Van Loon et al 1977; Borison & Diamond 1979; Euvrard et al 1980; Hruska et al 1980; Perry et al 1981; Arnauld et al 1981; Di Paolo et al 1981; Menniti & Baum 1981). However, what we do suggest is that the alteration in c.n.s. drug concentrations following the changed hormonal status likely plays a role in some, if not many, of these observations.

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