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# Hypophysectomy does not prevent increased cerebral dopamine turnover following sulpiride administration

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Hypophysectomy is claimed to prevent increased forebrain dopamine turnover produced by administration of sulpiride. We have measured the increase in dopamine metabolite concentrations caused by sulpiride following surgical removal of the pituitary. In saline-treated control animals and in hypophysectomized rats 1 week or 1 month following surgery, administration of sulpiride caused marked elevations of striatal, nucleus accumbens and tuberculum olfactorium, homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations which were maximal 4–8 h following drug administration. The maximal increases in nucleus accumbens and tuberculum olfactorium were generally comparable in control and hypophysectomized animals, except for a greater increase in HVA levels in the nucleus accumbens 1 month following hypophysectomy. However, maximal increases in HVA and DOPAC in striatum were more pronounced in hypophysectomized rats 1 week or 1 month following surgery compared with control animals. At 30 min following sulpiride administration only inconsistent changes in dopamine turnover were observed in both control and hypophysectomized rats. Hypophysectomy does not prevent sulpiride from increasing forebrain dopamine turnover suggesting this is due to a direct interaction with cerebral dopamine receptors.

Sulpiride is an effective antipsychotic agent in man (Edwards et al 1980), but it exhibits atypical neuroleptic properties in animal experiments (see Jenner & Marsden 1981). In particular, sulpiride is a selective antagonist of adenylate cyclase independent dopamine receptors (Trabucchi et al 1975; Elliott et al 1977; Jenner et al 1978). The drug penetrates poorly into the central nervous system, so it is necessary to administer large doses to elicit its pharmacological effects in the brain. In contrast, sulpiride exerts a marked effect on the pituitary, which lies outside the blood–brain barrier complex, causing elevation of circulating prolactin levels (Mancini et al 1976; Muller et al 1979). Portaleone and colleagues suggested that hypophysectomy prevents the ability of sulpiride to alter forebrain dopamine turnover (Portaleone et al 1978), so they postulated that the central effects of the drug may be secondary to its action in the pituitary. Fuxe and coworkers have shown discrete changes in brain catecholamine turnover following systemic administration of prolactin (Fuxe et al 1977). However, sulpiride can act directly on forebrain dopamine receptors for it can displace radioactive ligands such as [$^3$H]spiperone from rat striatal membranes (Jenner et al 1978). In addition, direct injection of sulpiride into the nucleus accumbens potently inhibits the hyperactivity induced by the bilateral administration of dopamine into this area (Costall & Naylor 1976; Honda et al 1977).

In recent experiments, the administration of sulpiride peripherally has been shown to cause displacement of the in-vivo binding of [$^3$H]spiperone to various rat brain areas (Kohler et al 1979, 1981, unpublished observations). We have re-investigated the effect of hypophysectomy on the ability of sulpiride to cause an increase in cerebral dopamine turnover. In the original study by Portaleone and his colleagues, dopamine turnover was measured 0·5 h following administration of sulpiride (Portaleone et al 1978). In our previous studies we have been unable to find substantial change in turnover or behaviour so soon after drug administration (Elliott et al 1977), so in the present study we have examined the time course of drug effect.

## METHODS

Normal, sham-operated or hypophysectomized male Wistar rats (180 ± 30 g at the time of surgery; Charles Rivers Ltd) were obtained 2 days following hypophysectomy. The rats were housed in groups of 6 under conditions of standard lighting (12 h light/dark cycle) at 23 ± 2 °C. They received a glucose supplement in their drinking water and were given access to a rock salt preparation. At 1 week or 1 month following surgery animals received sulpiride

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(50 mg kg$^{-1}$ i.p.; Delagrange) or 0·9% sodium chloride solution (saline) (0·5 ml). Sulpiride was dissolved in a minimum quantity of 2% sulphuric acid and diluted to volume with distilled water, the pH being brought to neutrality by the addition of 1 M sodium hydroxide solution. Rats were killed at intervals up to 24 h following sulpiride administration, by cervical dislocation and decapitation. The brain was rapidly removed onto ice and the paired corpus striatum, tuberculum olfactorium and nucleus accumbens were dissected out and immediately frozen in liquid nitrogen. Regional concentrations of homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were determined using the extraction procedure of Earley & Leonard (1978) and the automated fluorimetric technique of Westerink & Korf (1977).

To determine the success of hypophysectomy the skulls from the animals used for determination of regional HVA and DOPAC content were placed in a decalcifying solution (formic acid 5%, formalin 5%, distilled water 90%) for 2 weeks, and the pituitary fossa and surrounding areas then were removed for histological examination and processed through wax; blocks including the pituitary fossa were cut at 12 μm. Haematoxylin and eosin stained sections were examined on a Zeiss photomicroscope II.

At 1 month following surgery, other normal, sham-operated or hypophysectomized animals received 0·9% NaCl (saline) (0·5 ml) or sulpiride (50 mg kg$^{-1}$ i.p.) and 3 h later (the time of maximal drug effect), were anaesthetized using ketamine (150 mg kg$^{-1}$ i.p.; Vetalar, Parke Davis) to avoid stress-induced elevation of prolactin levels. When anaesthetized the animals were decapitated, blood was collected into heparinized tubes, and plasma samples were separated from red blood cells by rapid centrifugation at 4 °C. The plasma samples were stored at −20 °C for subsequent assay of prolactin content.

Plasma prolactin concentrations were estimated in 10 μl aliquots, and were assayed in duplicate by an homologous double antibody radioimmunoassay system with kits provided by NIAMDD, Bethesda, Maryland, USA (Brown-Grant & ter-Haar 1977). Concentrations of prolactin were expressed as ng ml$^{-1}$ of respective RPI standards. The sensitivity of the assay was 7·5 ng ml$^{-1}$ of NIAMDD-RPI. The mean inter-assay coefficient of variation was 13% for prolactin in plasma pools with low concentrations of this hormone.

Statistical differences between the elevations in HVA and DOPAC occurring in sulpiride-treated animals compared with their respective saline-treated controls were examined using a two-tailed Student's *t*-test.

## RESULTS

Histological examination of the hypophysectomized animals showed the pituitary fossa was empty, apart from an occasional animal where scattered groups of adeno-hypophyseal cells were still present on the floor of the fossa. The pituitary was intact in those animals receiving sham-surgery. Hypophysectomy produced a fall in circulating prolactin levels below the level of detection of the assay (Table 1). Levels of circulating prolactin in sham-operated animals were not different from those found in control naive animals. Administration of sulpiride (50 mg kg$^{-1}$ i.p.) 3 h previously to sham-operated or control animals produced identical elevation of circulating prolactin concentration. The administration of sulpiride (50 mg kg$^{-1}$ i.p.) to hypophysectomized animals still induced a rise in prolactin concentration although this was very small in comparison with the effect in drug-treated sham-operated or normal animals.

Table 1. Plasma prolactin concentrations in rats 3 h following administration of sulpiride (50 mg kg$^{-1}$ i.p.) or 0·9% saline (0·5 ml) to normal, sham-operated or hypophysectomized animals 1 month following surgery.

| | Prolactin (ng ml$^{-1}$) | |
|---|---|---|
| | Saline | Sulpiride |
| Control | 31·7 ± 3·4 | 92·4 ± 12·2* |
| Sham-operated | 25·4 ± 7·3 | 101·2 ± 15·6* |
| Hypophysectomized | <7·5† | 17 ± 2 |

Values are the mean (± 1 s.e.m.) of duplicate determinations carried out on plasma samples from at least 6 animals in each group.
* $P < 0·05$ compared with saline-treated animals.
† $P < 0·05$ compared with control animals.

The basal levels of HVA and DOPAC in sham-operated and hypophysectomized animals were not different from those found in control animals either 1 week or 1 month following surgery. The mean (± s.e.m.) concentrations of dopamine metabolites in brain areas were as follows: HVA: striatum 910 ± 130 ng g$^{-1}$; nucleus accumbens 390 ± 50 ng g$^{-1}$; tuberculum olfactorium 690 ± 100 ng g$^{-1}$. DOPAC: striatum 1920 ± 170 ng g$^{-1}$; nucleus accumbens 1800 ± 100 ng g$^{-1}$; tuberculum olfactorium 1760 ± 80 ng g$^{-1}$.

Measurement of HVA and DOPAC levels in the striatum, nucleus accumbens and tuberculum olfactorium in the 24 h period following administration of

Table 2. Concentrations of HVA and DOPAC in the tuberculum olfactorium (TUO), nucleus accumbens (NA) and striatum (ST) of control and hypophysectomized rats following administration of sulpiride (50 mg kg$^{-1}$ i.p.).

| Time after sulpiride administration | Pre-treatment | % of values for saline-treated control animals HVA TUO | HVA NA | HVA ST | DOPAC TUO | DOPAC NA | DOPAC ST |
|---|---|---|---|---|---|---|---|
| 30 min | None | 160 ± 31 | 203 ± 31* | 153 ± 21 | 183 ± 20* | 190 ± 20* | 161 ± 20* |
| | Hypophysectomy (1 week) | 117 ± 16 | 110 ± 31 | 121 ± 9 | 165 ± 16* | 154 ± 9* | 157 ± 8* |
| | Hypophysectomy (1 month) | 148 ± 19 | 362 ± 98* | 188 ± 37* | 130 ± 17* | 173 ± 22* | 146 ± 19* |
| Maximal effect (4–8 h) | None | 266 ± 49* | 249 ± 49* | 276 ± 52* | 271 ± 28* | 226 ± 43* | 271 ± 34* |
| | Hypophysectomy (1 week) | 207 ± 24* | 295 ± 46* | 503 ± 31*† | 207 ± 15* | 269 ± 29* | 440 ± 20*† |
| | Hypophysectomy | 230 ± 22* | 448 ± 67*† | 486 ± 67*† | 194 ± 13*† | 236 ± 13* | 400 ± 15*† |

Results are expressed as the mean (± s.e.m.) of at least 6 determinations and as a percentage of values obtained in saline-treated control animals.
* $P < 0.05$ sulpiride compared with saline-treated animals.
† $P < 0.05$ hypophysectomy compared with control animals.

sulpiride (50 mg kg$^{-1}$ i.p.) showed marked increases in the level of these dopamine metabolites in saline-treated control animals and hypophysectomized animals (Table 2; Fig. 1). At the time of maximal drug effect the changes in the tuberculum olfactorium and nucleus accumbens were generally comparable in control and hypophysectomized animals, with the exception of the HVA rise in the nucleus accumbens 1 month following hypophysectomy. However, increases in HVA and DOPAC in the striatum were greater in hypophysectomized animals 1 week or 1 month following surgery compared with control animals. In all cases the time of maximal drug effect (between 4–8 h) appeared unchanged.

Sulpiride (50 mg kg$^{-1}$ i.p.) 30 min previously caused variable changes in dopamine turnover (Table 2). Thus, DOPAC concentrations were elevated in all brain areas of both control animals or animals hypophysectomized 1 week previously. However, HVA levels were raised in the nucleus accumbens of control animals and in the nucleus accumbens or striatum of animals hypophysectomized 1 month earlier, but not elsewhere.

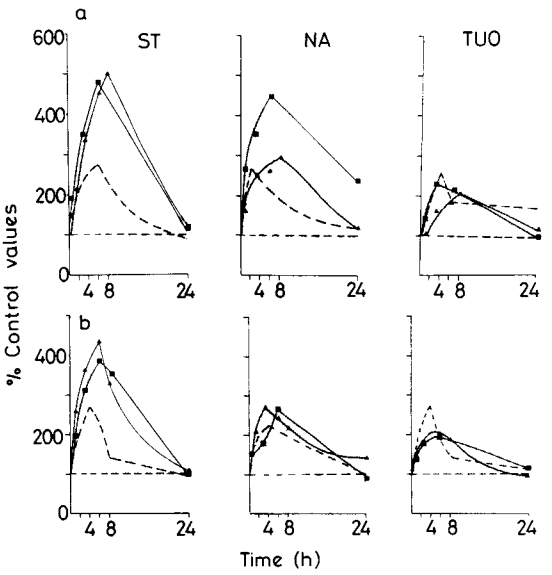


FIG. 1. Time courses of changes in the concentration of (a) HVA and (b) DOPAC in the striatum (ST), nucleus accumbens (NA) or tuberculum olfactorium (TUO) produced by administration of sulpiride (50 mg kg$^{-1}$ i.p.). In each group, the effect of sulpiride (50 mg kg$^{-1}$ i.p.) administered to control animals is shown by the shaded areas, which enclose the mean with 2 s.d. The effect of sulpiride (50 mg kg$^{-1}$ i.p.) in animals 1 week (—▲—) or 1 month (—■—) after hypophysectomy is shown.
The results are the mean of determinations carried out on tissues from at least 6 animals and expressed as a percentage of values obtained in saline-treated rats.
* $P < 0.05$ compared to saline-treated animals.

## DISCUSSION

The data show that in animals with successful removal of pituitary, sulpiride continues to cause increase in cerebral dopamine turnover as judged by the levels of the major metabolites HVA and DOPAC. Indeed, in the striatum at least, the increase may be larger in hypophysectomized animals than in control animals. This may be due to a breakdown of the blood-barrier caused by surgery allowing higher concentrations of sulpiride to enter the brain. Alternatively, altered hepatic drug metabolism might take place since pituitary function alters monooxygenase activity. However, this would be unlikely to effect sulpiride levels substantially since

the drug is excreted mainly unchanged. These results, contrast with those of Portaleone and his colleagues (1978) and do not support the notion that sulpiride acts to alter forebrain dopamine systems by a primary action at the pituitary. Our data are consistent with the view that sulpiride is a centrally-acting dopamine antagonist, albeit possessing atypical neuroleptic properties. The reason for the difference between our results and those of Portaleone and his colleagues (1978) is not certain. We would suggest, however, that one factor may be the short time after sulpiride administration that these workers employed to investigate changes in dopamine turnover. Changes in HVA and DOPAC concentrations were relatively small and inconsistent in our hands at 30 min following sulpiride administration. There is general agreement that the maximal effect of sulpiride occurs only 3 or more hours following drug administration. It is clear from the measurement of time course of effect of the drug on HVA acid DOPAC levels, that hypophysectomy does not prevent alterations in cerebral dopamine turnover caused by sulpiride.

Recently, Nicoletti & colleagues (1982) showed hypophysectomy to prevent increased glutamic and decarboxylase (GAD) activity in striatum and substantia nigra produced by administration of sulpiride (2 mg $kg^{-1}$ i.p.), a dose which in our hands has no effect on cerebral dopamine mechanisms. However, enzyme activity again was measured only 30 min after drug administration. Although serum prolactin levels were elevated at this time in intact rats, it is necessary to establish that the failure to elevate cerebral GAD activity in hypophysectomized animals applies to the entire time course of action of the drug.

The argument of Portaleone and his colleagues (1978) also hinged on the fact that previous studies failed to observe the penetration of sulpiride into brain (Benakis & Rey 1976). This work relied mainly on the distribution of $^{14}C$-labelled sulpiride of low specific activity. We suggest that this was not sufficiently sensitive to detect the small quantity of sulpiride which penetrates into the brain. Using highly labelled [$^3H$]sulpiride, we have found that radioactivity can be found in various areas of the rat brain (unpublished observations). Indeed, only small amounts of sulpiride need to penetrate into the brain to act there. The focal injection of sulpiride into the nucleus accumbens showed it to potently inhibit dopamine-mediated motor behaviour (Costall & Naylor 1976; Honda et al 1977). This is confirmed by the ability of systemically administered sulpiride to displace [$^3H$]spiperone from its specific binding sites in rat brain in in-vivo experiments (Kohler et al 1979, 1981 and unpublished observations).

If the pituitary were critically involved in the ability of neuroleptic drugs to alter forebrain dopamine function then domperidone, a compound with an extremely limited ability to penetrate into the brain, would be expected to produce indices of alteration of cerebral dopamine function on peripheral administration, but this is not the case (Costall et al 1979).

In conclusion, we find that hypophysectomy does not prevent sulpiride from increasing forebrain dopamine turnover in rats. It is unlikely that pituitary factors are essential for the cerebral actions of such drugs. These data are consistent with our recent finding that hypophysectomy does not *prevent* the development of cerebral dopamine receptor supersensitivity in response to either sulpiride or haloperidol (Jenner et al 1981). Neuroleptic drugs including sulpiride probably act directly on forebrain dopamine receptors to produce their central pharmacological actions.

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