Localization of a new neuroleptic in the pituitary gland of the rat

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Summary. The ^{14}C-labelled, substituted benzamide neuroleptic Tiapride has been found to accumulate transiently in the pituitary gland of the rat following a single dose, and a prolonged retention of the drug was observed in the pars intermedia.

Certain substituted benzamides, such as sulpiride and sultopride[1,2], may be classed amongst the neuroleptic drugs. Many neuroleptics have marked effects on the endocrine system, including the pituitary gland[3], and such effects have been described for both sulpiride[4,5] and sultopride[6]. Recent studies of a new substituted benzamide, Tiapride (N[diethylaminoethyl]-2 methoxy-5 methyl sulfonyl benzamide hydrochloride[7])[2], demonstrated that this compound also has endocrine activity, and so the distribution of ^{14}C-labelled Tiapride in the pituitary gland of the rat was investigated.

Materials and methods. The labelled Tiapride was prepared (CEA, Saclay) with ^{14}C in the carbonyl position and a specific activity of 20.45 μCi/mg. The compound was dissolved in physiological saline, and male and female rats of approximately 100 g b.wt were treated p.o., i.v. or i.m. at a dose rate of 50 mg/kg b.wt. Animals were sacrificed by rapid decapitation at various time intervals after drug administration.

Animals used in autoradiographic studies were dosed i.m. 2 were killed at each of the following times after administration: 15 min, 30 min, 1 h, 2 h, 6 h and 24 h, whilst untreated rats were used as control for chemography. Immediately after decapitation, the heads were quench-frozen in a mixture of acetone and dry ice, and embedded in aqueous 1% carboxymethylcellulose at −30 °C. Coronal sections 40 μm thick were cut using a Leitz freezing sledge microtome and were dried at −20 °C. Sections were then placed in contact with Kodak X-ray film at −20 °C for intervals varying from 2 days to 3 months, and films were developed using Kodak D19 developer.

Tissue analyses. For each of the 3 routes of administration, 6 animals (3 males and 3 females) were killed at 15, 60, 120 or 240 min after drug administration. Blood was collected at sacrifice, and 10–100 μl samples were solubilized for 1 h in 1.5 ml of a 1:1 mixture (v/v) of Soluene-100 (Packard) and isopropanol. The resulting solution was decolorized with 0.5 ml H_2O_2 at 30–35%, and was counted in a Beckman LS 233 liquid scintillation counter using a 9:1 mixture (v/v) of Insta-Gel (Packard) and 0.5 N HCl.

The pituitaries were weighed and dissolved in Soluene-350 (Packard), and ^{14}C-activity estimated by liquid scintillation counting using Dimilume-30 (Packard) as the scintillation fluid. 1 group (table) was used to examine distribution of activity in the pituitary by dissection of fragments on ice, with subsequent treatment of fragments being as described for whole pituitary.

Results. No attempt was made to distinguish between ^{14}C-labelled Tiapride and its labelled metabolites. Autoradiography revealed distinct pituitary accumulation of ^{14}C-activity from 15 min to 1 h after the i.m. administration, a marked reduction in activity after 2 h, very low activity at 6 h, and no evidence of residual activity at 24 h. These kinetics were supported by assays of whole pituitaries with similar results for all 3 routes of administration in males and females. The highest concentrations occurred at 15 min after i.v. administration with

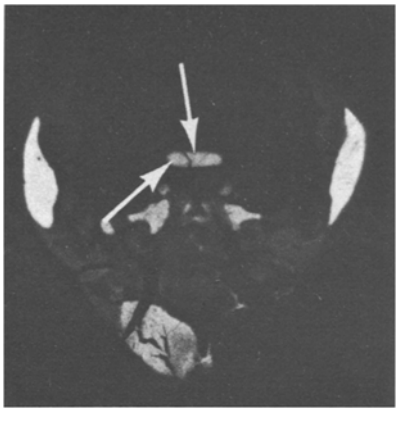

Fig. 1. Autoradiography of coronal section of head ♀ rat at level of pituitary 15 min after i.m. administration to demonstrate ^{14}C-Tiapride throughout the pituitary. ×1.5. (Upper arrow = neurohypophysis; lower arrow = adenohypophysis.)

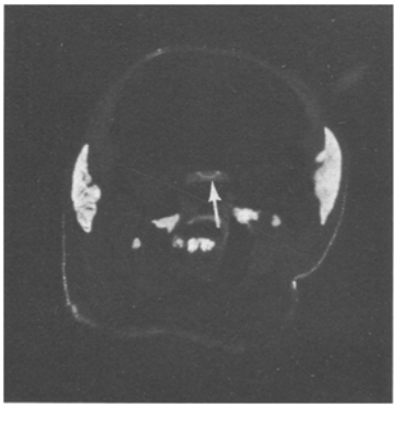

Fig. 2. Autoradiography of coronal section of head ♀ rat at level of pituitary 2 h after administration of ^{14}C-Tiapride. Note reduced concentration in pituitary and localization in the pars intermedia (arrow.) ×1.5.

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pituitaries containing a mean activity equivalent (± SD) to 67.73 ± 2.27 µg Tiapride/g fresh weight, the corresponding mean blood concentration (± SD) being 13.45 ± 0.74 µg/ml. Pituitary activities fell rapidly and were near blood levels (< 5 µg/ml) at 4 h after administration.

Assay of pituitary fragments (table) and autoradiography (figure 1) indicated an even distribution of Tiapride throughout the pituitary gland during the 1 h after administration. 1 h later, the activity in the neurohypophysis and adenohypophysis declined markedly, but there was some retention of label in the intermediate lobe (figure 2). The same distribution was observed until 6 h after drug administration, but with much diminished activity (autoradiograms required 3 months exposure) and at 24 h the localization was no longer detected. These studies also revealed the presence of ^{14}C in the parotid, submaxillary and sublingual salivary glands (figure 2) and the choroid plexus of the brain.

Pituitary and blood Tiapride levels based on ^{14}C-activity 30 min after an intramuscular injection of 50 mg/kg to female rats

Rat No.	µg Tiapride/g fresh tissue or ml of blood			
	Blood	Right lobe of adenohypophysis	Left lobe of adenohypophysis	Neurohypophysis / pars intermedia
1	15.10	57.57	55.56	64.51
2	12.10	52.46	49.98	56.48
3	10.49	48.55	47.15	51.92

Discussion. Several substituted benzamides including sulpiride have distinct effects on the mammary glands and genital tract of the rat[8], whilst sulpiride has no such effects in hypophysectomized male and female rats[9]. These observations suggest that these drugs have a direct effect upon the pituitary gland, and this is further supported by the finding that sulpiride and 2 other substituted benzamides, sultopride and metoclopramide, produce distinct histological changes in the adenohypophysis of the rat after prolonged treatment[8]. The present finding that Tiapride localizes in the pituitary gland is also consistent with the direct action of these drugs in this site.

The pars intermedia, which lies between the residual cleft and the neurohypophysis, is an endocrine gland, and much speculation has been reported concerning its function and significance in mammals[10]. It is believed to secrete a number of hormonal substances and to be associated with response to thirst[11], sodium depletion[12], and the experimental convulsion states produced by strychnine sulphate poisoning[13].

Thus the investigation of the action of Tiapride and possibly other substituted benzamides upon the above responses could throw some light upon the functions of pars intermedia.

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Methadone and brain development

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Summary. The effect of maternally administered methadone hydrochloride (5 mg/kg) on brain development of offspring treated during gestation and/or lactation was studied in 21-day-old rats. Animals treated during gestation or lactation were the most severely affected, with reductions in brain weights (12% and 30%, respectively), and DNA values (50% and 34%, respectively) observed.

Methadone is a synthetic narcotic analgesic that is commonly used in detoxification and maintenance programs for narcotic-addicted pregnant women[2]. Children delivered by methadone-exposed mothers often have retardation in body growth[3,4] and exhibit behavioral abnormalities[3]. Animal studies have shown that offspring maternally subjected to methadone may have congenital malformations of the central nervous system[5,6] and abnormal body growth[7–9]. The timing and duration of opiate treatment appear to be important factors in governing neurobiological response[9]. Rat pups maternally treated with methadone during gestation and/or lactation have altered patterns of brain development as determined by wet weight and macroscopic measurements[9]. At weaning (day 21), significant reductions in brain weights were only observed in those animals treated during either gestation or lactation. The present study was undertaken in order to ascertain if this impairment in brain growth is also accompanied by a reduction in neural cellularity.

Materials and methods. Female (180–200 g) Sprague-Dawley rats, housed under controlled conditions[8,9] with food and water ad libitum, were treated daily (8.00 h) with an i.p. injection of either 5 mg/kg dl-methadone hydrochloride (Dolophine, Eli Lilly Co., Indianapolis, Indiana) or an equivalent volume of physiologic saline. 5 days after the beginning of treatment, females were mated and sperm positivity indicated day 0 of gestation. Within 4 h after birth, litters of methadone-treated mothers were either transferred into cages with control mothers or placed with other methadone-injected mothers; these 2 groups of pups were considered to have been subjected to methadone during 'gestation alone' or given a combined 'gestation-lactation' treatment, respectively. A third group of pups delivered by control females were placed with methadone-injected mothers and were considered to be exposed to methadone during 'lactation alone'. Appropriate saline-injected 'controls' were included for each group. Litter size was maintained at 8 pups per mother, with an equal distribution of males and females.

All offspring were sacrificed by decapitation on day 21 and whole brains were removed and weighed. Brain tissues were homogenized in 1.4 M sucrose, and lipids extracted successively with chloroform-methanol (2:1,