EFFECTS OF INTRAVENTRICULAR INJECTIONS OF 6-HYDROXYDOPAMINE ON PLASMA LH AND FSH IN MALE RATS

J. H. KITCHEN, K. B. RUF AND E. V. YOUNGLAI

*Departments of Neurosciences and of Obstetrics and Gynecology,
McMaster University, Health Sciences Center, Hamilton,
Ontario L8S 479, Canada*

(Received 11th September 1973)

Summary. The involvement of central catecholaminergic neurons in tonic gonadotrophin release was investigated. Male rats were given a single cerebral intraventricular injection of 6-hydroxydopamine hydrochloride (6-OHDA; 170 μ g free base) dissolved in 0.001 N-HCl. Plasma LH and FSH were measured by double antibody radioimmunoassay. In the 6-OHDA-treated animals, LH was significantly reduced ($P < 0.001$) after 1 hr and remained consistently lower for 8 hr. After a transient elevation, the controls showed no significant change ($P > 0.01$). No difference in LH concentrations was found between experimental groups sampled at 2 days or later ($P > 0.01$). Levels of FSH were less consistently different. Administration of 6-OHDA dissolved in different vehicles, with varied pH and osmolarity, gave similar results which suggests that the quinone derivative of 6-OHDA is effective in producing the catecholaminergic impairment. These findings indicate that LH release in the male rat may be controlled (or at least modulated) by a central adrenergic mechanism.

INTRODUCTION

The hypothesis that hypothalamic catecholamines stimulate the release of pituitary LH and FSH now has considerable support (Schneider & McCann, 1969, 1970; Rubinstein & Sawyer, 1970; Kamperi, Mical & Porter, 1970, 1971). These publications suggest that brain amines, in particular dopamine, noradrenaline, or adrenaline, are involved in the regulation of LH and FSH release, and Schneider & McCann (1970) have suggested that this is achieved by hypothalamic dopaminergic neurons which synapse with the neurons that secrete the releasing hormone(s). A noradrenergic synapse may connect the preoptic area and the neurosecretory neurons located caudally (Kalra & McCann, 1973).

The dopamine analogue, 6-hydroxydopamine (6-OHDA), produces a partial destruction of, or, at lower doses, a transient functional impairment of, catecholaminergic neurons when injected intraventricularly (Uretsky & Iversen, 1970; Breese & Traylor, 1970; Ungerstedt, 1971; Jacks, De Champlain

& Cordeau, 1972), and is able to block ovulation in rats when administered during the previous oestrous cycle, presumably by preventing the LH surge (Kalnins & Ruf, 1971), and to induce prolonged periods of dioestrus (Kordon & Héry, 1971).

Central catecholaminergic control of male gonadal function is an area which is largely unexplored. The above findings led us to investigate the possibility that central catecholaminergic neurons may be involved in tonic gonadotrophin release.

MATERIALS AND METHODS

Animals

All animals were 70- to 80-day-old male Wistar rats maintained on a 12-hr light/12-hr dark cycle with unrestricted feeding.

Drug administration

The 6-OHDA (Roche) was dissolved in 0.001 N-HCl gassed with nitrogen. This solvent prevents immediate oxidation of 6-OHDA. Using stereotaxic coordinates, 170 µg 6-OHDA (free base $\equiv$ 250 µg HBr salt) dissolved in 20 µl solvent were injected into the anterior portion of the lateral ventricle of the brain at the rate of 20 µl/min. The control rats received the solvent alone. The surgical procedure, including ether anaesthesia, took approximately 10 min for each animal. The coordinates were periodically checked by X-ray using an injection of a radio-opaque dye ('Lipiodol', Denver Laboratories, Toronto).

Samples

The rats were lightly anaesthetized with ether and blood samples were obtained by heart puncture at 10, 15, 20 and 30 min, 1, 2, 3, 4, 5 and 8 hr and 2 and 3 days after injection. An intraperitoneal injection of saline (2 ml) was given after each blood sample (2 ml) was taken. Samples, usually two but occasionally three, were taken from each animal with at least 1 hr between cardiac punctures to minimize the combined stress of handling, anaesthesia and bleeding. When a third sample was taken, its haematocrit value was determined; and samples with a value <35% were discarded.

Radioimmunoassay

Plasma LH and FSH were measured in duplicate by a double antibody radioimmunoassay using NIAMD reagents. Bovine albumin or egg albumin (Sigma) were used as carrier protein. Purified rat LH and FSH were iodinated with carrier-free ^{131}I (Cambridge Nuclear Corp.) according to the NIAMD specifications. Duplicate standard curves were run with assays of more than 200 tubes. Plasma obtained from hypophysectomized, castrated and 'normal' male rats were included in each assay. The between-assay coefficient of variation was 8.1% for the LH, and 9.2% for the FSH assay. The within-assay coefficient of variation was 4.2% for the LH and 5.1% for the FSH assay. The limit of sensitivity was 15 ng/ml for LH and 25 ng/ml for FSH. The total volume of plasma in each tube was constant in every assay (200 µl) and plasma values

were determined from the steepest portions of the dose-response curves (between 15 and 85% of the counts precipitated).

Osmolarity and pH of intraventricular solvents

Two additional solvents, 0.85% saline containing 0.1% ascorbic acid and an artificial cerebrospinal fluid described by Merlis (1940), were used to determine the effect of variation in pH and in osmolarity of the solvent. Each solvent alone or with 6-OHDA was injected intraventricularly into four animals, the injected volume of fluid always being 20 μ l. Isotonicity was maintained in the Merlis solution by reducing NaCl. Blood was taken at 1 and 3 hr after injection and assayed for LH and FSH content. The 6-OHDA was dissolved at a concentration of 50 μ M in each of the three solvents and u.v. absorption spectra at 400 to 200 nm were determined on a Beckmann Acta III spectrophotometer (Saner & Thoenen, 1971). Each sample was scanned at room temperature at 2, 5, 10, 15 and 30 min, and 1 and 2 hr after preparation. A final scan was made 24 hr later.

Analysis of data

The data obtained were analysed for mean, standard error of the mean and *t* values, using the unpaired *t* test. A value of $P < 0.01$ was taken to be significant.

Histology

Two animals from each of the above groups were perfused through the heart with ice-cold 10% buffered formaldehyde after blood sampling and their brains were removed for histology. Serial coronal paraffin wax-embedded sections (10 μ m) were cut from the optic chiasma to the mamillary bodies and then stained with haematoxylin and eosin.

RESULTS

Concentrations of LH and FSH

As shown in Text-fig. 1, LH concentrations (mean $\pm$ S.E.M.) in the rats treated with 6-OHDA were significantly reduced ($P < 0.001$) after 1 hr (40.4 ± 2.0 ng/ml) as compared to those in controls (63.0 ± 2.5 ng/ml) and remained consistently lower at 2 hr (43.1 ± 4.2 ng/ml), 3 hr (33.9 ± 1.3 ng/ml), 4 hr (31.3 ± 3.5 ng/ml), 5 hr (36.1 ± 4.9 ng/ml) and 8 hr (32.0 ± 2.9 ng/ml). The controls, after an initial elevation and subsequent drop, showed no significant change ($P > 0.01$) during this period of time. Samples taken at 2 days or later showed no significant difference in LH concentrations ($P > 0.01$) between groups (Text-fig. 1). The concentrations of FSH in the treated animals were not significantly different from those in the controls at all intervals tested ($P > 0.01$), except at 5 hr and 2 days (Text-fig. 2), i.e. over most of the period when the LH values showed the most striking and consistent differences between experimental and control groups, the FSH values were unaffected.

Effects of pH and osmolarity of intraventricular solvents

The 6-OHDA remained stable for at least 2 hr when dissolved in 0.001 N-

TEXT-FIG. 1. Plasma LH concentrations in male rats following injection of (▲) 6-hydroxydopamine or (○) control solvent. Vertical bars represent mean $\pm$ S.E.M.; the numbers by each point denote the number of samples collected. Asterisks denote statistically significant differences ($P < 0.01$).

TEXT-FIG. 2. Plasma FSH concentrations in male rats following injection of (▲) 6-hydroxydopamine or (○) control solvent. Vertical bars represent mean $\pm$ S.E.M.; the numbers by each point denote the number of samples collected. Asterisks denote statistically significant differences ($P < 0.01$).

Table 1. The effect of cerebral intraventricular injections of different solvents on plasma LH and FSH in male rats

Solvents	pH	Osmolarity (mOsmol)	LH		FSH	
			1 hr after injection (Mean $\pm$ S.E.)	3 hr after injection (Mean $\pm$ S.E.)	1 hr after injection (Mean $\pm$ S.E.)	3 hr after injection (Mean $\pm$ S.E.)
Solvents alone						
0.001 N-HCl	2.85	18	55.8 $\pm$ 4.0 (4)	67.0 $\pm$ 1.2 (4)	280.0 $\pm$ 35.5 (4)	302.5 $\pm$ 20.1 (4)
0.85% NaCl containing 0.1% ascorbic acid	2.87	290	60.0 $\pm$ 2.0 (4)	59.0 $\pm$ 4.5 (4)	270.0 $\pm$ 16.5 (4)	286.3 $\pm$ 20.2 (4)
Artificial cerebrospinal fluid	7.61	320	60.0 $\pm$ 4.5 (4)	63.8 $\pm$ 3.8 (4)	301.3 $\pm$ 13.9 (4)	297.5 $\pm$ 17.3 (4)
6-OHDA + solvents						
6-OHDA + 0.001 N-HCl	2.38	96	40.3 $\pm$ 2.2 (4)	32.3 $\pm$ 2.6 (4)	256.3 $\pm$ 41.3 (4)	258.8 $\pm$ 35.0 (4)
6-OHDA + 0.85% NaCl with 0.1% ascorbic acid	2.35	373	37.0 $\pm$ 2.0 (4)	30.0 $\pm$ 2.1 (4)	298.8 $\pm$ 8.8 (4)	323.8 $\pm$ 38.0 (4)
6-OHDA + artificial cerebrospinal fluid	6.15	320	30.5 $\pm$ 3.8 (4)	27.0 $\pm$ 1.2 (4)	315.0 $\pm$ 37.1 (4)	310.0 $\pm$ 40.5 (4)

Figures in parentheses represent the number of determinations.

HCl and in 0.85% saline containing 0.1% ascorbic acid. The absorption spectrum obtained at low pH (pH 3) represents the characteristics of unchanged 6-OHDA (peak at 290 nm). At pH 7.6, 6-OHDA was so rapidly oxidized that it was not possible to register the spectrum of the unchanged 6-OHDA (Saner & Thoenen, 1971). The peak of the quinone derivative (270 nm) was constant for 2 hr. Subsequently, another peak (220 nm) became apparent after 24 hr.

No significant change ($P > 0.01$) was seen between LH or FSH plasma concentrations in control animals receiving the different solvents at either 1 or 3 hr after injection (Table 1). Similarly, no differences were detected in the 6-OHDA-treated animals using the different solvents. The significant differences ($P > 0.001$) found between LH concentrations in controls and treated animals at 1 and 3 hr are shown in Table 1. No significant change in FSH was found (Table 1).

Histopathological changes

Intraventricular injection of 6-OHDA in the frontal horn of the lateral ventricle resulted in dilatation of the ventricle with some destruction or detachment of the ependyma near the injection site. Infiltration of neuronal tissue by mononuclear phagocytes was occasionally seen adjacent to the ependyma. Damage to the ependyma appeared most severe in the rats injected with artificial cerebrospinal fluid/6-OHDA. In all cases, however, the ependymal lining of the third ventricle was intact. No ependymal or tissue damage was noted with the solvents alone, but 0.001 N-HCl and 0.85% saline containing 0.1% ascorbic acid caused some dilatation in the lateral ventricle near the injection site.

DISCUSSION

A wide variety of pharmacological approaches has been used in recent years to investigate central neural involvement in gonadotrophin secretion and release. Drugs which reduce central catecholaminergic neural activity have generally been found to suppress gonadotrophin release. Conversely, adrenergic agents exert largely stimulatory effects, although discrepant results are reported in the literature. Most of the research on central adrenergic control has been concerned with female reproduction, in particular ovulatory mechanisms which involve a separate and distinct 'surge centre', thought to exist in the preoptic region of the hypothalamus (for review, see Coppola, 1971). The male has been comparatively neglected although studies have shown that intraventricular infusions of dopamine alter gonadotrophin release suggesting the possibility of a tonic central catecholaminergic control (Kamberi *et al.*, 1970, 1971).

The present studies indicate that tonic LH release in the male rat may indeed be regulated by a central catecholaminergic mechanism. Reduction of about 50% of normal plasma LH concentrations was achieved after an intraventricular injection of 250 μ g 6-OHDA (salt). It is interesting that the plasma LH concentrations of 6-OHDA-treated animals revert to normal 2 or 3 days after injection. This suggests (i) recovery of the functionally impaired neurons, (ii) a compensatory reduction in the steroid-sensitivity of certain synapses, or

(iii) the development of supersensitivity by the denervated or partly denervated neurons. Functional recovery following the administration of 6-OHDA has also been observed with respect to the oestrous cycle (Kordon & Héry, 1971) and the central control of ACTH secretion (Ganong, Scapagnini, Cuello & Shoemaker, 1973).

The present investigation shows that either 6-OHDA or its quinone derivative injected intraventricularly can produce a functional impairment which is manifested as a decreased plasma LH concentration. The osmolarity or pH of the solvent does not seem to affect greatly the mechanism of action, possibly since the volume injected (20 μ l) is small relative to the volume of the ventricular system.

Although the results obtained for FSH do not show the reduction found in LH, the possibility that tonic release of FSH may also be centrally controlled by a catecholaminergic mechanism cannot be excluded. If FSH-releasing hormone is synthesized at sites between the preoptic and arcuate-ventromedial regions implicated in the LH-releasing hormone (Motta, Piva, Tima, Zanisi & Martini, 1971), a diffusion problem may exist with intraventricular injections and the 6-OHDA may not have reached sufficient intraneuronal concentration to produce functional impairment in catecholaminergic neurons involved in FSH release. Further work is in progress involving intracerebral injections which will help to resolve this problem and give further insight into the specific catecholaminergic mechanism involved.

The dose of 6-OHDA used for these experiments is known to cause depletion of hypothalamic catecholamines (Uretsky & Iversen, 1970; Breese & Traylor, 1970). Although destruction of the catecholaminergic nerve terminals occurs with high or repeated doses of 6-OHDA (Bloom, Algeri, Groppetti, Revuelta & Costa, 1969; Ungerstedt, 1971), the catecholamine content with low doses (200 μ g) can be reduced without detectable ultrastructural changes (Bartholini, Thoenen & Pletscher, 1971). Brain concentrations of serotonin (5-HT) and γ -aminobutyric acid (GABA) are not significantly altered by 6-OHDA treatment (Bloom *et al.*, 1969; Uretsky & Iversen, 1970; Jacks *et al.*, 1972). The specificity of 6-OHDA for catecholaminergic neurons appears to be due to differences in the ability of the neurons to take up and concentrate 6-OHDA (Ungerstedt, 1971) and degeneration presumably depends upon the attainment of a critical intraneuronal concentration of either 6-OHDA itself (Malmfors & Sachs, 1968) or its oxidation products and metabolites. Saner & Thoenen (1971) postulated that the quinone of 6-OHDA could react with tissue protein nucleophilic groups to cause nerve degeneration. Alternatively, H_2O_2 produced by auto-oxidation of 6-OHDA has been considered the causative factor in neuronal degeneration (Heikkila & Cohen, 1972). Recent work has demonstrated that the open-chain quinone is the major initial product of oxidation of 6-OHDA and the zwitterionic form of this compound is the main chromophore in neutral pH solutions (Powell & Heacock, 1973). The slow rate of cyclization of the open-chain quinone probably indicates that this compound, rather than cyclized products, has the greatest physiological significance (Powell & Heacock, 1973). It must be pointed out that the selectivity and specificity of action of 6-OHDA (Bloom *et al.*, 1969; Uretsky & Iversen, 1970;

Breese & Traylor, 1970; Bartholini *et al.*, 1971; Ungerstedt, 1971) has been questioned by Poirier, Langelier, Roberge, Boucher & Kitsikis (1972) who feel that neuronal degeneration is due to non-specific destruction caused by the generation of H_2O_2 . The histology included in this study demonstrated only slight histopathological changes in the immediate area of the injected lateral ventricle which suggests that 6-OHDA may indeed be considered as a valuable tool for neuroendocrine research. Non-specificity appears to be present only in those regions exposed to a high local concentration of 6-OHDA.

ACKNOWLEDGMENTS

The technical assistance of Mr M. J. Holmes was greatly appreciated. This work was supported by a grant to the Developmental Neurobiology Group of the Medical Research Council of Canada. Thanks are extended to the National Institute of Arthritis and Metabolic Diseases (NIAMD) for radio-immunoassay reagents.

REFERENCES

- BARTHOLINI, G., THOENEN, H. & PLETSCHER, A. (1971) Biochemical effects of 6-hydroxydopamine in brain without detectable ultrastructural changes. In *6-Hydroxydopamine and Catecholamine Neurons*, p. 163. Eds. T. Malmfors and H. Thoenen. North-Holland Publishing Co., Amsterdam.
- BLOOM, F. E., ALGERI, S., GROPPETTI, A., REVUELTA, A. & COSTA, E. (1969) Lesions of central nor-eppinephrine terminals with 6-OHDA dopamine: biochemistry and fine structure. *Science, N.Y.* **166**, 1284.
- BRESE, G. R. & TRAYLOR, T. D. (1970) Effect of 6-hydroxydopamine on brain norepinephrine and dopamine. Evidence for selective degeneration of catecholamine neurons. *J. Pharmac. exp. Ther.* **174**, 413.
- COPPOLA, J. A. (1971) Brain catecholamines and gonadotropin secretion. In *Frontiers in Neuroendocrinology* 1971, p. 129. Eds. L. Martini and W. Ganong. Oxford University Press, New York.
- GANONG, W., SCAPAGNINI, U., CUELLO, A. & SHOEMAKER, W. (1973) Effect of 6-hydroxydopamine (6-OHDA) on ACTH secretion. *Proc. 55th Ann. Meeting Endocrine Society, Chicago*, Abstract No. 66.
- HEIKKILA, R. & COHEN, G. (1972) Further studies on the generation of hydrogen peroxide by 6-hydroxydopamine. *Molec. Pharmacol.* **9**, 241.
- JACKS, B. R., DE CHAMPLAIN, J. & CORDEAU, J. P. (1972) Effects of 6-hydroxydopamine on putative transmitter substances in the central nervous system. *Eur. J. Pharmac.* **18**, 353.
- KALNINS, I. & RUF, K. B. (1971) Blocage de l'ovulation chez la rate par la 6-hydroxydopamine. *Schweiz. med. Wschr.* **101**, 1124.
- KALRA, S. P. & McCANN, S. M. (1973) Effect of drugs modifying catecholamine synthesis on LH release induced by preoptic stimulation. *Endocrinology*, **93**, 356.
- KAMBERI, I. A., MICAL, R. S. & PORTER, J. C. (1970) Effect of anterior pituitary perfusion and intraventricular injection of catecholamines and indoleamines on LH release. *Endocrinology*, **87**, 1.
- KAMBERI, I. A., MICAL, R. S. & PORTER, J. C. (1971) Effect of anterior pituitary perfusion and intraventricular injection of catecholamines on FSH release. *Endocrinology*, **88**, 1003.
- KORDON, C. & HÉRY, M. (1971) Effect of selective pharmacological destruction of central catecholaminergic (CA) neurons and of *l*-dopa administration on ovulation in the rat. *Physiologist, Wash.* **14**, 174, Abstr.
- MALMFORS, T. & SACHS, C. (1968) Degeneration of adrenergic nerves produced by 6-hydroxydopamine. *Eur. J. Pharmac.* **3**, 89.
- MERLIS, J. K. (1940) The effect of changes in the calcium content of the cerebrospinal fluid on spinal reflex activity in the dog. *Am. J. Physiol.* **131**, 67.
- MOTTA, M., PIVA, F., TIMA, L., ZANISI, M. & MARTINI, L. (1971) Intrahypothalamic localization of the nuclei synthesising the gonadotropin releasing factors. *J. neuro-visc. Rel.*, Suppl. **10**, 32.
- POIRIER, L. H., LANGELIER, P., ROBERGE, A., BOUCHER, R. & KITSIKIS, A. (1972) Non-specific histopathological changes induced by the intracerebral injection of 6-hydroxydopamine. *J. neurol. Sci.* **16**, 401.

- POWELL, W. S. & HEACOCK, R. A. (1973) The oxidation of 6-hydroxydopamine. *J. Pharm. Pharmac.* **25**, 193.
- RUBINSTEIN, L. & SAWYER, C. H. (1970) Role of catecholamines in stimulating the release of pituitary ovulating hormone(s) in rats. *Endocrinology*, **86**, 988.
- SANER, A. & THOENEN, H. (1971) Contributions to the molecular mechanism of action of 6-hydroxydopamine. In *6-Hydroxydopamine and Catecholamine Neurons*, p. 265. Eds. T. Malmfors and H. Thoenen. North-Holland Publishing Co., Amsterdam.
- SCHNEIDER, H. P. G. & MCCANN, S. M. (1969) Possible role of dopamine as transmitter to promote discharge of LH-release factor. *Endocrinology*, **85**, 121.
- SCHNEIDER, H. P. G. & MCCANN, S. M. (1970) Mono- and indoleamines and control of LH secretion. *Endocrinology*, **86**, 1127.
- UNGERSTEDT, U. (1971) Histochemical studies on the effect of intracerebral and intraventricular injections of 6-hydroxydopamine on monoamine neurons in the rat brain. In *6-Hydroxydopamine and Catecholamine Neurons*, p. 101. Eds. T. Malmfors and H. Thoenen. North-Holland Publishing Co., Amsterdam.
- URETSKY, N. J. & IVERSEN, L. L. (1970) Effects of 6-hydroxydopamine on catecholamine containing neurons in the rat brain. *J. Neurochem.* **17**, 269.