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The effect of 6-hydroxydopamine on the oestrus cycle and fertility of rats

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Increases in the numbers of foetuses resorbed and stillborn are reported in rats after treatment with 6-hydroxydopamine given before and during pregnancy. 6-Hydroxydopamine (100 mg kg^{-1} i.p.) given before mating had no effect on the oestrus cycle. Intrahypothalamic 6-hydroxydopamine (200 μg) produced a temporary loss of oestrus. However, after mating the injected rats had normal pregnancies despite substantially lower levels of brain noradrenaline.

Noradrenaline is found in substantial quantities throughout the female genital tract. In the ovaries it is localized within sympathetic nerves and the ovaries seem to be responsible for maintenance of normal noradrenaline levels in the remainder of the tract (Owman & Sjöberg, 1973).

It has long been known that surgical sympathectomy can cause abnormal pregnancies in rats (Bacq, 1932) and increase the number of stillborn in cats (Simone & Ross, 1938). The effects of chemical sympathectomy by a selective destruction of adrenergic nerve endings with 6-hydroxydopamine (6-OHDA) (Tranzer & Thoenen, 1968; Thoenen & Tranzer, 1968) on the oestrus cycle and fertility of mice have been examined by Airaksinen, Castrén & others (1972) and Castrén, Airaksinen & others (1973). Since the ovarian sympathetic innervation in mice is poorer than in rats, in this study we report changes in fertility after 6-OHDA intraperitoneally and changes in oestrus after intrahypothalamic injection of 6-OHDA to rats.

Catecholamines within the central nervous system are considered to be important in the regulation of oestrus by influencing the release of hypothalamic regulating hormones. 6-OHDA does not easily cross the blood-brain barrier in mature rats but when injected into the brain or cerebrospinal fluid it will lower central noradrenaline and dopamine levels. Intraventricular 6-OHDA has been shown to inhibit oestrus in mature rats (Kalnins & Ruf, 1971) and block ovulation induced by pregnant mares serum (Hökfelt & Fuxe, 1972).

METHODS

Young adult female rats, random bred from Sprague-Dawley origin (Orion Oy, Helsinki), initially 200–250 g, were housed in groups of three or four during the examination of the oestrus cycle. This was checked daily for two weeks and those rats not showing a regular incidence of oestrus were eliminated from the experiment. After the examination of oestrus, one male rat was introduced to each cage. Seven days later the males were changed to ensure pregnancies occurred.

6-OHDA (2,4,5-trihydroxyphenethylamine, Hässle Ab.) was dissolved immediately before use in a solution of ascorbic acid (1 mg ml^{-1}) in 0·9% NaCl. Rats receiving peripheral injections were initially given 100 mg kg^{-1}, intraperitoneally weekly, and the same weekly dose was administered subcutaneously after mating to avoid damage to the foetuses.

Rats injected intrahypothalamically were mildly anaesthetized with pentobarbitone sodium (25 mg kg^{-1}) and injected once according to Valzelli (1964) with either 200 or 100 μg of 6-OHDA or solvent alone in a volume of 10 μl. The accuracy of the injection was checked with small quantities of dye. Normally most dye remained within the lower hypothalamus but a small amount did go intraventricularly, through the injection channel.

Rats receiving peripheral injections were decapitated after term and the brains, hearts and ovaries removed for assay. The number of resorptions was counted as the number of placental sites minus the number in each litter, live and stillborn. Those rats receiving 6-OHDA intrahypothalamically were decapitated a few days before the expected date of delivery and the same organs dissected. The tissues were stored at −20°. Noradrenaline was determined according to Miller, Cox & Snodgrass (1970) as modified by Karppanen, Airaksinen & Särkimäki (1973).

Student's *t*-test was used to investigate the significance of a difference between mean values. *P* values less than 0·05 were considered significant.

RESULTS

Effect of 6-OHDA on the oestrus cycle

In those rats receiving 6-OHDA intraperitoneally, after two doses of 100 mg kg^{-1} there were no significant changes in the oestrus cycle. All the animals in this group became pregnant.

Table 1. *Effect of intraperitoneal and intrahypothalamic injections of 6-hydroxydopamine on the oestrus cycle.*

Intraperitoneal injection	Number of animals in group	Number with interrupted oestrus	%
Control	7	0	0
6-OHDA (100 mg kg^{-1})	7	1	14
Intrahypothalamic injection			
Control	6	1	16
6-OHDA (200 μg)	10	7	70*
6-OHDA (100 μg)	6	3	50

* = $P<0·05$.

Table 2. *Effect of intraperitoneal and intrahypothalamic injections of 6-hydroxydopamine on the fertility and noradrenaline content in brains, hearts and ovaries.*

Intraperitoneal injection	Number in group	Average fertility			Noradrenaline concn (μg g^{-1})		
		Live	Stillborn	Resorbed	Brains	Hearts	Ovaries
Control	7	9·2±1·4	0·0	1·4±0·6	0·37±·07	0·82±·08	0·87±·14
6-OHDA(100 mg kg^{-1})	7	2·0±0·9**	3·4±1·5*	6·3±1·6**	0/37±·05	0·25±·06***	0·48±·09*

Intrahypothalamic injection	Number in group	Living foetuses	Resorbed	Whole Brains	Hypothalamus	Hearts
Control	7	10·5 ± 1·0	0·9 ± 0·7	0·31 ± ·01	0·89 ±·07	0·88 ± ·15
6-OHDA(200 μg)	10	8·7 ± 0·9	1·2 ± 0·6	0·19 ± 0·3**	0·23±·03***	0·96 ± ·08
6-OHDA(100 μg)	4	10·5 ± 1·3	0·0	0·36 ±·05	—	—

Results are expressed as means ± s.e. * = $P<0·05$: ** = $P<0·01$ *** = $P<0·001$.

Intrahypothalamic injections of 200 μg and, to a lesser extent, 100 μg had an inhibitory effect on oestrus (Table 1). The oestrus cycle was disturbed for between one to two weeks and its return corresponded with the disappearance of other side-effects of the treatment, such as anorexia, catatonia and asymmetric circling behaviour (Bloom, Algeri & others, 1969; Laverty & Taylor, 1970). One of the control rats lost the next oestrus, probably due to the known effects of pentobarbitone on the oestrus cycle (Everett, Sawyer & Markee, 1949; Wutke, Gelato & Meites, 1972). Two rats in the group receiving 100 μg of 6-OHDA and one in the 200 μg group did not become pregnant.

Effect of 6-OHDA on fertility

After the completion of the oestrus cycle investigation, the rats were mated and 21–26 days later they gave birth. The number of live births was significantly decreased in the group receiving 6-OHDA peripherally and the numbers of stillborns and resorptions correspondingly increased (Table 2). Those rats treated intrahypothalamically before mating did not differ significantly from the saline controls in either the litter size of the numbers stillborn or resorbed.

The noradrenaline content of hearts and ovaries was reduced after peripheral 6-OHDA but, as expected, there was no change in brain noradrenaline levels (Table 2). Rats treated intrahypothalamically had normal noradrenaline levels in hearts and ovaries but whole brain noradrenaline was reduced to 55% of controls and to 25% in sections corresponding to the hypothalamus.

DISCUSSION

The neuronal control of hypothalamic regulating hormones is a subject of some controversy. Whether dopamine stimulates the release of hypothalamic gonadotrophic regulating hormones FSH-RH/LH-RH (Schneider & McCann, 1970; McCann, Kalra & others, 1972) or inhibits it (Fuxe & Hökfelt, 1970), or whether there is a dual control with 5-hydroxytryptamine (Zolovic & Labhsetwar, 1973), is uncertain.

Our results with 6-OHDA, although producing a distinct interruption of oestrus, are difficult to interpret. The higher dose (200 μg), although interrupting more than one cycle, was incapable of producing a permanent loss of oestrus in all but one rat. It is well known that dopaminergic nerves are less sensitive to the effects of 6-OHDA than noradrenergic nerves (Bloom & others, 1969; Uretsky & Iversen, 1970) particularly those in the median eminence (Jonsson, Fuxe & Hökfelt, 1972). Perhaps the dose used was only capable of inducing a functional impairment of transmission without degeneratory changes occurring in the neurons.

The changes in fertility observed after peripheral injections seem to substantiate the reports that surgical sympathectomy induced abnormal pregnancies in rats and cats. The early literature on this subject is conflicting, suggesting that differences were related to the completeness of sympathectomy and/or the extent of damage to non-sympathetic nerves. However, 6-OHDA is selectively taken up into adrenergic nerve endings causing neuronal destruction even when given by routes other than intravenously (Goldman & Jacobowitz, 1971).

The present results are in agreement with those for mice reported by Castrén, Airaksinen & others (1973). In their work the number of placental sites was not always counted but, in retrospect, it seems that the smaller litter size was due to an increase in the number of offspring resorbed.

The exact mode of action of 6-OHDA has not yet been elucidated. Some may cross the placenta and lower noradrenaline levels in the foetus as was observed in mice. The low number of viable offspring in the 6-OHDA-treated group make significant comparisons difficult. However there is no evidence to suggest that deprivation of the sympathetic system is incompatible with life, since rats sympathectomized at birth are capable of reaching maturity (Thoenen, 1972).

Another site of action may be in the sympathetic nerves of the placental blood vessels which are responsible for the nutrition of the foetus. Vasoconstrictor tone may be progressively lost, with some foetuses receiving a disproportionate share of the maternal blood while those suffering a deficiency will die and be resorbed.

There is also an interaction between oestrogens and sympathetic nerves (Owman & Sjöberg, 1973). At any of these three points 6-OHDA may be acting to produce its abortive effect.

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