EFFECTS OF INTRAVENOUS CUPRIC SULPHATE ON HYPOPHYSIAL ACTIVITY IN THE FEMALE RAT

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Summary. In normal cyclic female rats, an injection of 16 mg CuSO_4 /kg induces pseudopregnancy (as determined by uterine traumatization) when given on the day of oestrus, or on the following day. Pentobarbital anaesthesia did not inhibit this effect of CuSO_4 . Pseudopregnancy, as determined by inhibition of the induction of vaginal cornification by exogenous oestrogen, was also induced by CuSO_4 in anovulatory females, provided the injection was given from 6 hr before, to 24 hr after injection of human chorionic gonadotrophin. The latter was necessary since CuSO_4 did not induce ovulation in forty experimentally produced anovulatory rats. Cupric sulphate injected on the day of vaginal pro-oestrus in normal females did not alter the incidence of spontaneous ovulation nor the number of ova shed. These data suggest an effect of copper ion in the rat on luteal regulatory mechanisms rather than on ovulatory mechanisms; the site of action cannot, however, be specified.

INTRODUCTION

In the oestrous rabbit, the intravenous injection of copper acetate induces both the ovulation of fertile ova (Suzuki & Bialy, 1964; Staples & Bialy, 1967) and pseudopregnancy (Fevold, Hisaw & Greep, 1936). Although a direct effect of copper ion on the pituitary has been proposed (Fevold *et al.*, 1936; Sawyer & Markee, 1950), recent experiments on the implantation of microgram quantities directly in the hypothalamus or pituitary suggest a neural site of action of copper in the rabbit (Hiroi, Sugita & Suzuki, 1965). The influence of this ion on the regulation of gonadotrophin secretion in the female rat, however, has not been investigated extensively. Dury & Bradbury (1941, 1943) demonstrated that copper injection induces pseudopregnancy only in the oestrous female. The present study confirms this observation in normal cyclic female rats. In addition, the occurrence of ovulation and/or pseudopregnancy following cupric sulphate injection has been recorded in experimentally anovulatory rats.

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METHODS

Sprague-Dawley rats were housed in a controlled environment of 14 hr of light and 10 hr of darkness per day. Daily vaginal smears were obtained at 08.00 hours and normal rats were required to exhibit three regular vaginal cycles in succession before selection for experimental use. A 1% unbuffered cupric sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) solution in physiological saline was prepared; the volume of solution injected was determined by the dose of cupric ion/body weight of the recipient rat.

In cyclic females, pseudopregnancy was established by the decidual reaction to uterine traumatization produced by looping two pieces of thread through the antimesometrial wall of the uterus on the 4th day of a continuing dioestrus. The rats were killed 5 days after uterine traumatization and the presence or absence of deciduomata was recorded. Pseudopregnancy was also investigated in female rats rendered anovulatory (determined by laparotomy and the persistence of vaginal cornification) by exposure to constant illumination. Ovulation was induced by the injection of 10 i.u. human chorionic gonadotrophin (HCG) and CuSO_4 was injected at varying times before or after HCG. In order to avoid possible alterations in uterine capacity to develop deciduomata in rats in persistent vaginal oestrus, progesterone secretion was detected by treating the females daily with oestradiol benzoate, 1 μg , for 5 days beginning 5 or 6 days after HCG injection. Treatment with 1 μg oestradiol benzoate induces vaginal cornification in the spayed female within 2 to 3 days. Females which did not display vaginal cornification in response to this treatment were therefore considered to be actively secreting progesterone (Jones & Astwood, 1942).

Attempts were made to induce ovulation in rats rendered anovulatory in several ways: twenty-nine were injected with 10 to 30 μg testosterone propionate on the 4th, 5th or 6th day of life; six were exposed to constant illumination; and five were subjected to electrolytic destruction of the preoptic region of the brain, or to anterior hypothalamic de-afferentation according to the technique described by Halász & Gorski (1967). Before experimentation, laparotomy was performed to confirm the absence of corpora lutea which was indicated by constant vaginal cornification. The presence of corpora lutea in these ovaries at laparotomy 7 days after CuSO_4 injection indicated that ovulation had occurred. Finally, CuSO_4 was injected into the tail vein of normal cyclic rats (without anaesthesia) at various times on the day of vaginal pro-oestrus. The influence of this treatment on spontaneous ovulation was ascertained by removing the oviduct the following morning, and counting the tubal ova by transillumination.

RESULTS

Pseudopregnancy studies

Since CuSO_4 is a toxic compound, the relationship between the lethal dose and the induction of pseudopregnancy was determined in eighty adult female oestrous rats (Table 1). Most of the rats given CuSO_4 at a dose of 18 mg/kg or more died within 24 hr. However, all the rats given not more than 16 mg/kg

survived. In addition, 16 mg/kg was sufficient CuSO_4 to induce pseudo-pregnancy in all oestrous rats, whereas less than 12 mg/kg did not induce pseudopregnancy. The 16 mg/kg dose was used in subsequent experiments.

The data of Table 2 indicate that CuSO_4 given on the day of vaginal oestrus or 24 hr later was highly effective in inducing pseudopregnancy as judged by uterine decidual reactivity. However, CuSO_4 given on the day of dioestrus or early pro-oestrus was essentially without effect. The very high

TABLE 1
MORTALITY AND PSEUDOPREGNANCY RATE AFTER CuSO_4
INJECTION IN NORMAL CYCLIC FEMALES ON THE DAY OF
VAGINAL OESTRUS

Dose of CuSO_4 (mg/kg)	No. of rats injected	No. of deaths	No. of rats showing pseudopregnancy*	% of surviving rats showing pseudopregnancy
5	10	0	0	0
10	10	0	0	0
12	10	0	4	40
14	10	0	6	60
16	10	0	10	100
18	10	5	5	100
20	10	7	3	100
22	10	10	0	—

* Determined by decidual reaction after uterine traumatization.

TABLE 2
RATE OF PSEUDOPREGNANCY* FOLLOWING CuSO_4 INJECTION AT DIFFERENT STAGES OF
THE OESTRUS CYCLE

Treatment	Stage of vaginal cycle at injection				
	Di-oestrus 08.00 hours	Pro-oestrus		Oestrus 08.00 hours	Post-oestrus 08.00 hours
		08.00 hours	17.00 hours		
CuSO_4 injection†	1/16‡	2/18	5/14	18/18	12/15
Nembutal§ and CuSO_4 injection	0/15	0/11	3/12	14/14	11/14
Nembutal	0/14	0/10	0/12	0/12	0/12

* Determined by decidual reaction after uterine traumatization.

† 16 mg/kg.

‡ Number pseudopregnant/number injected.

§ 32 mg/kg given 20 min before CuSO_4 .

incidence of pseudopregnancy when CuSO_4 was injected on the day of oestrus, or the following day, was not altered when the rats were anaesthetized with pentobarbital (Nembutal) shortly before copper injection (Table 2).

In female rats displaying anovulatory persistent vaginal oestrus due to constant environmental illumination, CuSO_4 did not induce ovulation (see below). Therefore, in order to study the ability of copper ion to induce pseudo-pregnancy in these animals, it was necessary to induce ovulation with HCG. In this way it was possible to vary the time of injection of CuSO_4 in relation to the time of HCG injection (Table 3). When copper was injected within 24 hr

after HCG injection almost all rats continued in vaginal di-oestrus in spite of daily oestrogen injection. When this metallic ion was injected 48 hr after, or 12 hr before HCG, pseudopregnancy was not observed consistently.

TABLE 3

RATE OF PSEUDOPREGNANCY* FOLLOWING HCG AND CuSO_4 ADMINISTRATION AT VARIOUS TIMES IN THE ANOVULATORY RAT UNDER CONSTANT ILLUMINATION

	HCG	CuSO_4	HCG, then CuSO_4 after indicated interval (hr)								$\text{Na}_2\text{SO}_4^\dagger$	Na_2SO_4 and HCG
			-24	-12	-6	0	+6	+12	+24	+48		
No. pseudo-pregnant/no. injected	0/9	0/6	0/6	2/6	4/6	5/6	5/6	5/6	4/6	0/6	0/6	0/6
% Showing pseudopregnancy	0	0	0	33	67	83	83	83	67	0	0	0

* Determined by the persistence of a di-oestrous vaginal smear in spite of daily injection of 1 μg oestradiol benzoate.

† In these studies, the injection of Na_2SO_4 (16 mg/kg) was utilized as a control procedure.

Ovulation studies

In normal females. The data presented in Table 4 demonstrate that CuSO_4 injected at any of five different times on the day of vaginal pro-oestrus had no effect on the number of ova released by each ovulating rat. Nor was there any marked effect on the incidence of ovulation among these animals.

In anovulatory adult females. Cupric sulphate was injected into various preparations of anovulatory persistent oestrous rats: twenty-nine androgenized rats, three rats with a preoptic-anterior hypothalamic lesion, two rats after surgical transection of ovulatory neural pathways, and six rats housed under continuous illumination. Cupric sulphate did not induce ovulation in any of these forty rats.

TABLE 4

INCIDENCE OF OVULATION, AND NUMBER OF OVA SHED IN THE NORMAL RAT FOLLOWING INJECTION OF CuSO_4 OR OF Na_2SO_4 AT VARIOUS TIMES ON DAY OF PRO-OESTRUS

Treatment		Time on day of vaginal pro-oestrus				
		06.00	08.00	10.00	12.00	16.00
Na_2SO_4 injection (16 mg/kg)	Incidence of ovulation*	5/5	5/5	5/5	5/5	5/5
	Average no. of ova/ovulating rat	11.4	11.0	11.4	12.0	12.6
CuSO_4 injection (16 mg/kg)	Incidence of ovulation	9/10	7/10	5/5	5/5	5/5
	Average no. of ova/ovulating rat	10.9	9.9	11.8	13.2	11.4

* No. rats ovulating/no. injected.

DISCUSSION

In the rabbit, corpora lutea appear to function following ovulation whether ovulation is induced by sterile mating, exogenous gonadotrophin or copper ion

(Everett, 1961). In the rat, however, ovulation and CL activation appear to be regulated separately (Everett, 1961). The present data demonstrate a rather selective action of intravenous CuSO_4 on luteal function (presumably through hypophyseal luteotrophin). Although progesterone secretion was readily induced by CuSO_4 injections in oestrous cyclic females, and within 24 hr of an injection of HCG in anovulatory rats which had been exposed to constant illumination, ovulation was not induced by CuSO_4 in anovulatory females.

The confirmation that pseudopregnancy is induced only at oestrus supports the view that oestrogen facilitates this action of copper ion (Dury & Bradbury, 1941, 1943). On the other hand, the lack of effect of CuSO_4 12 hr before or 48 hr after HCG injection, and the studies of Nikitovitch-Winer & Everett (1958), suggest that the presence of CL capable of responding to the hypophyseal luteotrophin may also be a decisive factor. The observation that pentobarbital does not prevent the action of CuSO_4 recalls the controversy about the site of action of copper ion in the rabbit. Barbiturates can block the ovulatory stimulus in the immature (Quinn & Zarrow, 1964; Wagner & Brown-Grant, 1965) or adult female (Everett & Sawyer, 1950), and prevent the action of exogenous androgen in the neonatal female rat (Arai & Gorski, 1968). In either case, however, the relevant site of action of the barbiturate is unknown. Therefore, although the lack of an effect of pentobarbital suggests that CuSO_4 may act directly on the pituitary, one cannot dismiss the possibility that CuSO_4 acts on a terminal step in the neural mechanism distal to pentobarbital. In addition, it is possible that the action of CuSO_4 persists beyond the duration of effective anaesthesia.

Although CuSO_4 does not induce ovulation in the rat as in the rabbit, these negative findings are not surprising considering the inhibitory influence that the hypothalamus exerts over luteotrophin secretion in the rat (Everett, 1961). In fact, a non-specific action of copper ion which depresses hypothalamic function might be expected to induce pseudopregnancy, and also block ovulation. This is not the case, however, since CuSO_4 administration at different times on the day of vaginal pro-oestrus did not interfere with the neural stimulus for ovulation or its effectiveness, since both the incidence of ovulation and the number of ova shed were unaltered.

Therefore, although we cannot specify whether CuSO_4 inhibits hypothalamic function or directly stimulates pituitary secretion, its effects appear to be rather specific to a luteotrophin system. The lack of ovulation argues against a non-specific direct stimulatory effect on the pituitary, and the occurrence of normal ovulation in pro-oestrous rats argues against a non-specific inhibition of the hypothalamus.

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