OVULATION IN RABBITS FOLLOWING INTRAVENOUS AND INTRACEREBRAL ADMINISTRATION OF COPPER SULPHATE

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ABSTRACT

The present authors (1965) previously reported that the site of action of copper sulphate in the induction of ovulation might be in the vicinity of the posterior median eminence (PME) in the hypothalamus. The authors have now injected a solution of copper sulphate intravenously into adult female Japanese mongrel rabbits and also infused the solution directly into the PME of the hypothalamus. The 50% effective dose for inducing ovulation in some of the rabbits of the tested group was 0.76 μg/kg of copper sulphate in the PME infusion or 3.0 mg/kg in the intravenous injection. This means that the 50% effective dose of copper sulphate required for infusion into the PME is 1/4000 of the 50% effective dose required by intravenous injection.

Since Fevold et al. (1936) induced ovulation by the intravenous injection of copper salt in female rabbits, the copper ovulation has been used as a technique for elucidating the complex mechanism of ovulation. Accordingly, many experiments have been performed. Opinions, however, are divided about the site of action of copper salt associated with ovulation: Sawyer & Markee (1950) stated that copper salt may act directly on the anterior pituitary gland, and Suzuki (1961) reported that copper salt may act directly on the hypothalamus of the diencephalon. The present authors (Hiroi et al., in press) observed the effect on rabbit ovulation by implanting not more than 100 μg of copper salt into the various areas of the hypothalamus, into the various parts of the limbic system, and also into the anterior pituitary gland. Ovulation was thus found to occur only when the copper salt had been implanted into the vicinity of the posterior median eminence (PME). In the present studies, the minimal dose of
copper salt was determined that could induce ovulation by intravenous injection or by infusion into the PME.

MATERIALS AND METHODS

The animals used in the experiments were adult female Japanese mongrel rabbits weighing from 2000 to 3500 g and averaging 2480 ± SE 30 g, each maintained in individual cages for not less than 3 weeks under the same condition. Altogether 180 rabbits were used, excluding 9 rabbits in which the needle tip failed to reach the PME. Laparotomy was performed in all rabbits before the experiment to make sure that the ovaries were of normal size and that no ovulations were present. Each rabbit was injected with 85 µg of 17β-oestradiol (oestra-1,3,5(10)-triene-3,17β-diol) benzoate intramuscularly for 2 days in succession. 24 hours after the second injection, a physiological saline solution of copper sulphate (CuSO₄·5H₂O, reagent grade) was injected intravenously or infused into the PME. The animal was sacrificed by air-embolism 48 hours after the administration of copper sulphate; and the ovulated and haemorrhagic follicles in both ovaries were counted. The above mentioned injection or infusion of the copper sulphate solution was performed in the following way.

(1) Intravenous injection of the copper sulphate solution

The rabbits were divided into 5 groups for injection of 2.0, 2.5, 3.0, 3.5 and 4.0 mg per kilo gram of body weight, respectively, of a 1%/ (w/v) copper sulphate solution. The injection was performed slowly into the marginal ear vein. A control group was given 3 ml/kg of physiological saline solution. Each group consisted of 10 rabbits.

(2) Infusion of copper sulphate solution into the PME of the hypothalamus

The rabbit was anaesthetized with about 50 mg of thiopentone sodium (given via the marginal ear vein) and placed in a stereotaxic apparatus. A longitudinal skin incision of about 4 cm was made in the centre of the parietal region, and, by removing the periosteum from the bregma to the lamda, the skull completely exposed in that region. Then, following the map of Sawyer et al. (1954), the area chosen for puncture was bored with an electric drill, and a 28 gauge stainless steel tube was inserted into the PME. Subsequently, 0.005 ml of a 5%/ (w/v) to 0.003125 %/ (w/v) solution of copper sulphate was infused by means of a microsyringe. This infusion was done slowly over a period of not less than 10 minutes. For protection against post-operative infection, penicillin (10000 U) was injected daily for two successive days. The site of infusion was confirmed after sacrificing the rabbit, by reconstructing the serial sections of the brain. Only those rabbits in which the needle tip was found in the PME were chosen for evaluation of the effect of the infusion. For control, 0.005 ml of a physiological saline solution was similarly infused into the PME. Ten or more than ten rabbits were used in each group until the needle tips were confirmed by reconstruction, to have reached the PME in ten rabbits.

RESULTS

(1) Groups injected intravenously with copper sulphate solution

The ovulation rates that followed the intravenous injection of physiological

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saline solution used as control and of different doses of copper sulphate solution are given in Fig. 1.

**Fig. 1.**
Ovulation rates and total numbers of ovulations following intravenous injection of various doses of copper sulphate. (Each group consists of ten rabbits).

**Fig. 2.**
Ovulation rates and total numbers of ovulations following infusion of 0.005 ml of copper sulphate solutions into the posterior median eminence. (Each group consists of ten rabbits except the 2.0 % group which consists of nine does and the 5.0 % group which consists of seven rabbits).
(2) Groups infused with copper sulphate solution into PME of the hypothalamus

The rates of ovulation following physiological saline solution as control and of the different doses of copper sulphate solution into the PME of the hypothalamus are presented in Fig. 2. Three out of ten rabbits died in the 5.0% solution infusion group and one out of ten in the 2.0% group. The dead does have been excluded from Fig. 2. Destructive effects in the hypothalamus were not observed by histological examination, when lower concentrations of copper sulphate solution were infused into the hypothalamus. The dose capable of inducing ovulation in one half of ten rabbits is calculated to be 0.038% (w/v) (0.76 μg/kg) and is 3.0 mg/kg in the intravenous injection group.

DISCUSSION

Fevold et al. (1936) reported that a single dose of 10 to 15 mg of copper acetate injected into the marginal ear vein of the rabbit induced ovulation while Emmens (1940) reported ovulation with 10 mg of copper acetate, 30 mg of copper sulphate, or 25 mg of copper alanine. No accurate determinations of the doses required for inducing ovulation by intravenous injection having been found in previous papers, the present authors have carried out precise determinations of the required doses.

On the other hand, Brooks et al. (1940) reported that section of the stalk blocked copper-induced ovulation. Harris (1941) induced ovulation by infusing 0.05 mg of copper salt into the rabbit-third ventricle which was from 1/200 to 1/300 of the amount injected intravenously. Sawyer & Markee (1950) brought about ovulation by infusing 0.15 mg into the anterior pituitary gland and Tsuno (1957) by infusing 0.01 mg of copper salt into the periphery of the third ventricle. But no report on ovulation following systemic infusion of copper salts solutions into the hypothalamus was found.

Previously the authors (Hiroi et al., in press) had reported that by implanting copper salt in the different intracranial regions, the site associated with ovulation may be in the vicinity of the PME in the hypothalamus. Accordingly, minute amount of copper sulphate solutions were infused into the above site. The 50% effective dose of copper sulphate required for infusion into the PME is 1/3947 of the 50% effective dose required for intravenous injection for inducing ovulation. Thus, the ratio of the minimal intracranial infusion dose to the intravenous dose is very different between the previous and the present workers. This difference can be explained by the fact that the previous workers' intracranial infusions of copper salt were performed into the areas distant from the nerve nucleus which is the actual site of action of copper salt, and, consequently, that no ovulation could be observed unless fairly high doses were
infused. In contrast, the authors' infusion was directly into the hypothalamic area where copper salt seems to exert its effect, so that even the very low doses of copper salt administered could induce ovulation.

The results of the authors' experimental studies described in this paper further support their previous report that copper salt may exert its action in the vicinity of the PME.

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