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THE RELATION BETWEEN UROGASTRONE AND GONADOTROPHINS

BY

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A commercial preparation of chorionic gonadotrophin inhibited gastric acid secretion induced by carbachol in rats. When the preparation was incubated for 10 min at 100° C and *p*H 7, its gonadotrophic activity, as measured by a simple quantal assay based on the male toad spermiation response, was reduced by 92%. The inhibition of gastric acid secretion by the preparation was not significantly reduced by this treatment. It is concluded that the two activities are separable.

Early work on the inhibition of gastric acid secretion by urinary extracts followed observations that both the secretion of gastric acid and the incidence of peptic ulceration were lower in pregnant women than in non-pregnant women of the same age (Borodenko, 1909; Nakai, 1925; Sandweiss, Saltzstein & Farbman, 1939). It was possible that the inhibiting substance, called urogastrone by Gray, Culmer, Wieczorowski & Adkison (1940), was closely related to chorionic gonadotrophin although urogastrone has been demonstrated in the urine of both males and non-pregnant females (Gray, Wieczorowski & Ivy, 1939; Friedman, Recknagel, Sandweiss & Patterson, 1939; Necheles, Hanke & Fantl, 1939). The methods for the extraction of urogastrone (Gray, Wieczorowski, Wells & Harris, 1942; Friedman *et al.*, 1939; Wick, Medz & Pecka, 1949; Huff, Risley & Barnes, 1950; Mongar & Rosenoer, 1962) are similar to those used for chorionic gonadotrophin, and it has been found repeatedly that preparations of chorionic gonadotrophin inhibit gastric secretion (Culmer, Atkinson & Ivy, 1939; Friedman & Sandweiss, 1941; Code, 1951). Huff *et al.* (1950) showed that even highly purified gonadotrophins possessed this action. However, it was unlikely that the antisecretory activity was in the gonadotrophin molecule itself. They found that gonadotrophin preparations of different stages of purity and varying four-fold in gonadotrophic activity produced about the same degree of secretory inhibition. Whereas most of the gonadotrophic activity was destroyed by heating and by amylase digestion, the antisecretory activity was retained. Ghosh (1959) reinvestigated this problem using a new quantitative method of assay for inhibitors of gastric secretion, based on the continuous perfusion of the rat stomach. Two purified gonadotrophin preparations gave different activity ratios when assayed for gonadotrophic activity on the prostatic weight of rats and for antisecretory effects on gastric secretion induced by histamine.

Since the chromatographic procedure for the preparation of urogastrone in bulk described by Mongar & Rosenoer (1962) was developed from a modification of the procedure of Johnsen (1955) for the extraction of urinary gonadotrophins, it has become important to differentiate the urogastrone so prepared from chorionic gonadotrophin.

METHODS

Gonadotrophic assays. The male toad spermiation technique (Thorborg & Hansen, 1951) was used. This consists of counting the number of animals which spermiate in a given time after the injection of a standard or a test solution of gonadotrophin. Male toads (*Bufo bufo*) weighing 20 to 40 g were used in all the experiments. The animals were washed, dried and weighed. Standard and test solutions of gonadotrophins were injected through the buccal cavity into the ventral lymph sac, care being taken to avoid the heart and aorta. If the skin was punctured, the animal was not further used. The toads were placed in clean numbered jars containing 5 ml. of distilled water, and kept for 3 to 3.5 hr in a darkened room. The jars were then shaken gently and two samples of the water in each were examined for spermatozoa. Since the presence of even one sperm was recorded as a positive result, every negative sample was examined by at least two observers to eliminate counting errors. The results were recorded as a quantal response. In most of the experiments sixty toads were used in randomized groups of six ; three doses of standard gonadotrophin and of test sample were given in random order, thereby obtaining ten replications of each dose. The concentrations were adjusted so that each dose was in a volume equivalent to 1 ml./10 g of body weight ; the standard preparation of chorionic gonadotrophin was given in doses of 0.72 to 2.88 μg/ml./10 g of body weight (1 to 4 i.u./ml./10 g). Urogastrone and gonadotrophin were made up in 0.067 M-sodium phosphate buffer at *p*H 7.

Animals used in one experiment were not used again for 10 to 14 days. In a few early experiments the cloacae were washed out and small balloons tied in to collect all the urine passed after the injection of gonadotrophin. However, it was found that this elaborate method of preparation of the animal had little advantage over the former method described.

Urogastrone assays. The procedure was based on the technique for the continuous perfusion of the stomach of an anaesthetized rat maintained at 30° C (Rosenoer & Schild, 1962). The *p*H of the effluent perfusate was measured by a continuous-flow glass electrode and registered by a recording *p*H meter. By giving small doses of urogastrone 5 min before the stimulating dose of 1 μg of carbachol, and by interposing one recovery dose of carbachol alone, it was possible to give up to four doses of urogastrone to each animal. 2+2 assays were carried out using at least two animals for each assay. The limits of error of the quantitative assays were calculated by the method of Schild (1942) and of the quantal assays by the method of angular transformation. In the text the limits for the 5% level of probability are given in brackets after the mean result of the assay.

Preparation of urogastrone. This has been fully described elsewhere (Mongar & Rosenoer, 1962). Briefly, the active material was adsorbed on a zeolite column and differentially eluted with a buffered ammonium acetate-ethanol solution. One batch of freeze-dried material prepared from approximately 500 l. of urine was used throughout this study, and is referred to in the text as " urogastrone." Doses are given in terms of the weight of this preparation.

Chorionic gonadotrophin. This was chorionic gonadotrophin B.N. 436–030, 1,386 i.u./mg (Paines & Byrne).

RESULTS

Differential assays were carried out to compare the gastric antisecretory and gonadotrophic activities of the urogastrone preparation and of the commercial preparation of chorionic gonadotrophin. 1 mg of urogastrone was equivalent to

1.85 mg ($P=0.95$, 1.12 to 3.0 mg) of chorionic gonadotrophin by the rat gastric antisecretory assay. Fig. 1 shows the results of experiments in which the sperming effect of urogastrone injected into the ventral lymph sac of male toads was compared with

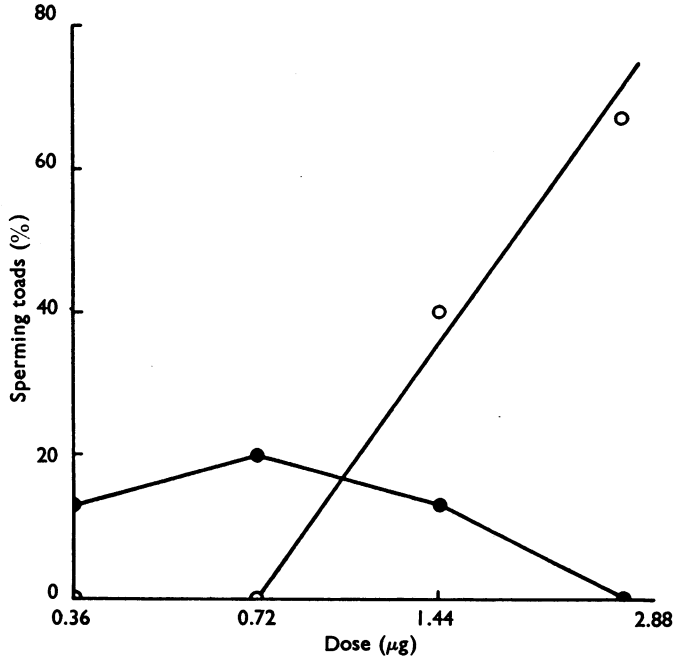

Fig. 1. Assay of urogastrone for gonadotrophic activity. The percentages of toads which spermed after injection of urogastrone (●) and after chorionic gonadotrophin (○) are plotted against the dose in μg of dry material/10 g of toad. Each point represents the mean for a group of fifteen toads.

that of commercial chorionic gonadotrophin. A negative log dose-response curve for urogastrone was found. A possible explanation is that the urogastrone preparation had both gonadotrophic and antigonadotrophic activities. Fig. 2 shows the results of an experiment in which a fixed dose of chorionic gonadotrophin was injected into the ventral lymph sac of the toads with various doses of urogastrone. No significant difference could be detected in the responses obtained and it was concluded that the urogastrone did not contain appreciable antigonadotrophic activity. There is thus no apparent explanation of the occasional positive results obtained with the urogastrone preparation in the spermiation assay.

To differentiate further between urogastrone and chorionic gonadotrophin a series of experiments was carried out to compare their gastric antisecretory and gonadotrophic activities after boiling. Incubation of urogastrone at *p*H 7.0 and 100° C for 10 min did not destroy its power to inhibit gastric secretion ; 101% ($P=0.95$, 76 to 134%) of the activity was retained after this treatment. After incubation of chorionic gonadotrophin under the same conditions 94% ($P=0.95$, 75 to 120%) of its gastric antisecretory activity was still present. When the incubated chorionic gonadotrophin was assayed with male toads the gonadotrophic activity was reduced by 92% ($P=0.95$, 86 to 96%) (Fig. 3).

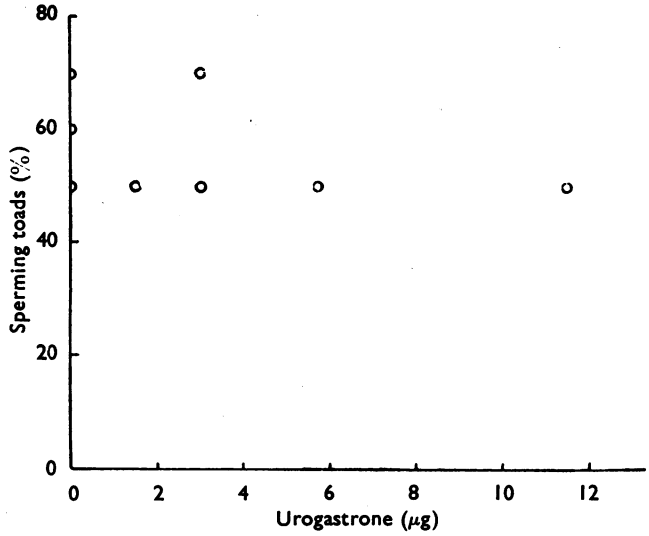

Fig. 2. Assay of urogastrone for antigonadotrophic activity. Chorionic gonadotrophin (2.9 μg/10 g of toad) was given with various doses of urogastrone (abscissa). The percentage of toads which spermed is plotted against the dose of urogastrone in μg/10 g of toad. Each point represents the mean for a group of ten toads.

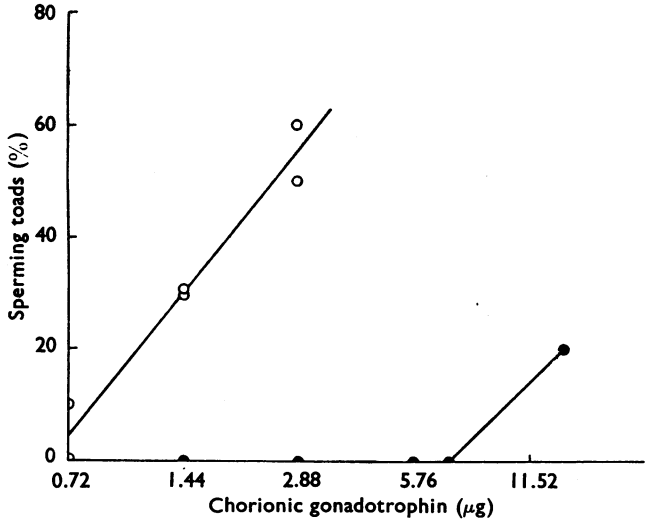

Fig. 3. Heat lability of chorionic gonadotrophin. The percentage of toads which spermed after injection of chorionic gonadotrophin is plotted against the dose in μg/10 g of toad for untreated (○) and for boiled (●) chorionic gonadotrophin. Each point represents the mean for a group of ten toads.

DISCUSSION

These experiments clearly differentiate the gonadotrophic and urogastrone activities of the preparations studied. The urogastrone activity of a commercial preparation of chorionic gonadotrophin was heat stable whilst its gonadotrophic

activity, as detected by spermiation in the male toad, was reduced by about 92% of control values by boiling for 10 min at *p*H 7, thus confirming the observations of Huff *et al.* (1950) and Ghosh (1959). Further evidence that urogastrone and gonadotrophin are not identical substances is provided by the finding that preparations of urogastrone extracted by the method of Mongar & Rosenoer (1962) had little gonadotrophic activity. This was unexpected since the chromatographic procedure used closely resembles that of Johnsen (1955) for the preparation of chorionic gonadotrophin. The urogastrone so prepared was therefore examined for antigonadotrophic activity. Such activity has recently been described in the urine of children (Soffer, Futterweit & Salvaneschi, 1961) and might mask any gonadotrophic activity. However, no evidence of such activity was obtained in experiments in which a fixed dose of chorionic gonadotrophin was injected together with various doses of urogastrone.

The question of the identity of the gastric antisecretory principle in commercial samples of chorionic gonadotrophin, previously studied by Ghosh (1959), and the urogastrone prepared by the method of Mongar & Rosenoer (1962) is unsolved. Both substances inhibit gastric secretion of acid induced by carbachol in the rat and both are heat stable, but their identification would require parallel quantitative assays in different species and further chemical purification.

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