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ACTIONS OF VASOPRESSIN-RELATED PEPTIDES ON GLYCOGEN METABOLISM IN THE PERFUSED RAT LIVER

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Abstract Both [8-lysine] vasopressin and oxytocin inhibited glycogen accumulation in the perfused liver from starved rats, at concentrations of 30–1000 pg/ml and 20–900 ng/ml respectively. [1-Deamino-8-D-arginine] vasopressin caused glycogenolysis in the perfused liver from fed rats over the concentration range 5–100 ng/ml. These effects resemble those previously reported for [8-arginine] vasopressin; they are discussed with reference to the potency of action of vasopressin-related peptides on the liver.

The hepatic actions of the hormones of the posterior pituitary gland have not been fully elucidated. Both vasopressin [1, 2] and oxytocin [1, 3] can cause glycogenolysis in the liver. In the case of [8-arginine] vasopressin at least, stimulation of glycogen breakdown and gluconeogenesis and inhibition of glycogen synthesis, in the perfused rat liver, is seen at concentrations which can occur in the intact animal, especially during haemorrhagic shock [4, 5]. The purpose of the experiments reported here was to study the effects of vasopressin-related peptides on glycogen metabolism in the perfused rat liver. Oxytocin and [8-lysine] vasopressin were selected because of their physiological significance (the latter in a small group of animals, eg. the pig) and [1-deamino-8-D-arginine] vasopressin (DDAVP) in view of its use in patients with diabetes insipidus [6].

EXPERIMENTAL

Perfusions of rat liver. Male rats of the Sprague–Dawley strain, weighing about 180 g, had free access to a standard diet of rat cubes and to water. Starvation was for 48 hr from about 10.00 hr. Rats were anaesthetised with diethyl ether–air, and liver perfusion was carried out with 50 ml of Krebs–Ringer bicarbonate containing albumin and washed rat erythrocytes [7]. Glucose was added to the perfusions so that the initial concentration was 5 mM in fed rat perfusions, and 28 mM when starved animals were used. In the latter group of perfusions gluconeogenic substrates (combined concentration 10 mM) were also added and were then infused (at 3 ml/hr from 15 min after the start of perfusion) in a mixture containing 0·5 M-sodium lactate, 0·33 M-glycerol and 0·17 M-sodium pyruvate [4]; net glycogen synthesis was followed in sequentially removed liver samples [7]. Peptides were added after various times, and caused no change in flow rate (about 2 ml/min/g, measured by drop-counting).

Chemicals and analytical methods. Chemicals were of the highest grade commercially available. L-lactic acid was from Sigma (London) Chemical Co. (Kingston, Surrey, U.K.) and sodium pyruvate from C. F. Boehringer Corp. (London, W.5, U.K.). [8-Arginine] vasopressin, which is the natural form of vasopressin in the rat, was obtained in solution from Sigma: Grade VI, a synthetic vasopressin of activity 360 units/mg. [8-Lysine] vasopressin, was also from Sigma: Grade IV, essentially oxytocin free, prepared synthetically and obtained in a powder form of activity 70–100 units/mg. [1-Deamino-8-D-arginine] vasopressin was from Fering A.B. Ltd. (Malmo, Sweden).

Glucose was measured by a glucose oxidase method [8] and glycogen was determined as glucose, after hydrolysis with fungal glucosidase [9].

RESULTS

Effect of posterior pituitary hormones on net glycogen synthesis. In the perfused liver from starved rats glycogen synthesis was measured under optimal conditions, in which circulating gluconeogenic precursors serve as carbon source and glucose is required in a regulatory capacity, to direct hexose phosphates into glycogen [7]. In these conditions [8-arginine] vasopressin inhibits glycogen synthesis [4]. Both [8-lysine] vasopressin and oxytocin also inhibited glycogen accumulation (Fig. 1); [8-lysine] vasopressin inhibited synthesis extensively at concentrations as low as 200 pg/ml (50 μunits/ml; pure hormone having a vasopressin-like activity of 250 units/mg when assayed by rat antidiuresis [10]), whereas 40–900 ng/ml of oxytocin were required for a significant effect to be obtained (20–400 m units/ml; pure hormone having an oxytocin-like activity of 450 units/mg [10]).

It should be noted that since peptides were added four times (each time to the concentration indicated), these data do not exactly reveal the concentration-dependence of peptide effects. However, [8-arginine] vasopressin does not accumulate in these conditions (Dr. Mary Forsling: personal communication), so the concentrations shown probably correspond closely to the 'average' concentration during perfusion.

Stimulation of glycogen breakdown by [1-deamino-8-D-arginine] vasopressin (DDAVP). During perfusion of livers from fed rats the glucose concentration in the medium reached a steady concentration of 8–12 mM within 50 min (Fig. 2). If DDAVP was then added in a single dose to the medium there was an efflux

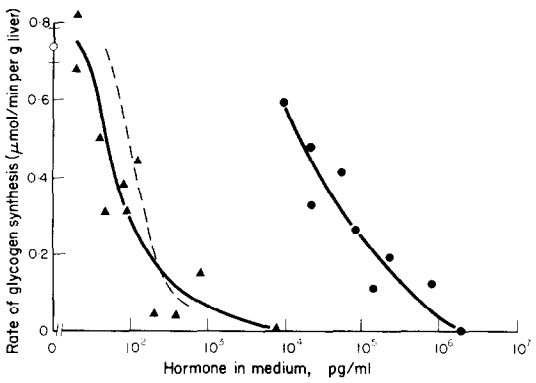

Fig. 1. Influence of oxytocin and [8-lysine]vasopressin on glycogen accumulation in the perfused liver of starved rats. Livers were perfused with gluconeogenic precursors and 28 mM glucose as described in the text; net glycogen synthesis was measured between 20 and 50 min [4, 7]. Both hormones were added at 10 min intervals, from 10 min after the start of the perfusion, to the concentration indicated (abscissa). For comparison, the concentration-dependence of the [8-arginine]vasopressin effect is included: broken line, see ref. [4]. Results are from individual perfusions except for controls [14]: open circle, bar gives S.E.M.): oxytocin (●), [8-lysine]vasopressin (▲).

of glucose (Figs. 2 and 3) as with [8-arginine] vasopressin [4]. This efflux was not as large as that obtained with [8-arginine] vasopressin [4]; this was not due to lack of liver glycogen as a second addition of DDAVP with [8-arginine] vasopressin caused a more marked glucose efflux (Fig. 2). This result showed that DDAVP does not inhibit [8-arginine] vasopressin action.

The extent of the increment in glucose concentration was dependent on the initial concentration of DDAVP over the range 5–100 ng/ml (Fig. 3, in which the dose response to [8-arginine] vasopressin [4] has been included for comparison: pure [8-arginine] vasopressin has a vasopressin-like activity of 400 units/mg [10]). The liver was much less sensitive to DDVAP than to vasopressin (Fig. 3).

DISCUSSION

The above experiments show that a number of vasopressin-related peptides can cause liver glycogenolysis (or prevent glycogen synthesis, which presumably reflects the same hormonal action), as has been established for [8-arginine] vasopressin [4, 5]. Although arginine-vasopressin is the natural form of vasopressin in the rat both this and the lysine hormone act on glycogen accumulation over a similar concentration range, i.e. 60–300 pg/ml.

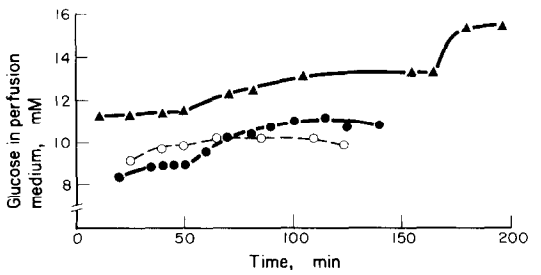

Fig. 2. Influence of DDAVP on the time-course of glucose output in the perfused liver of fed rats. Livers were perfused as described in the text. The following additions were made to the perfusion medium after 50 min: DDAVP at the following initial concentrations (ng/ml) 20 ○: 50 ● and 100 ▲. In the latter experiment a further 100 ng ml DDAVP and 20 ng/ml [8-arginine]vasopressin were added at 165 min. Results are representative single perfusions.

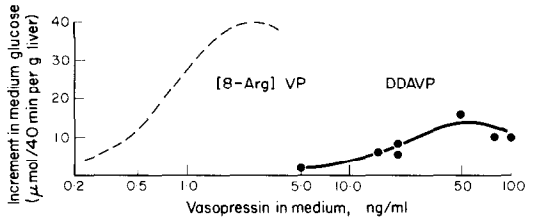

Fig. 3. Dependence on [8-arginine]vasopressin and DDAVP concentrations of stimulation of hepatic glucose output. Livers from fed rats were perfused as described in the text. After an initial steady glucose concentration was achieved, the hormones were added at various initial concentrations. Each point represents the increment in glucose output during the next 40 min, determined for each perfusion. The response to [8-arginine]vasopressin (broken line) is taken from ref. [4]; DDAVP (●).

The rat liver does not seem to be as sensitive to oxytocin or DDAVP as it is towards [8-arginine] vasopressin. The results obtained with oxytocin confirm those obtained *in vitro* by other workers [3]; Heidenreich [11] however found no oxytocin effect *in vivo* on the blood glucose concentration of the rat, perhaps as a result of the insensitivity of the liver to this hormone. Since massive doses of the hormone were required to inhibit glycogen accumulation, this action probably has no physiological significance.

A number of vasopressin and oxytocin analogues have been shown to produce hyperglycaemic responses, similar to that of the hormones themselves [12]. Since the vasopressin analogue DDAVP is administered to patients with diabetes insipidus [6] its effect on liver glycogenolysis was of interest. It caused liver glycogenolysis but was not as effective as [8-arginine] vasopressin. This is likely to be due to the D-arginine substitution, as [1-deamino] vasopressin retains its hyperglycaemic effect (as tested *in vivo*) [13].

Hepatic and vascular responses to vasopressin-related peptides may be compared. The two tissues exhibit (i) comparable sensitivity to [8-arginine] vasopressin (both responses being much less sensitive than that of the kidney), (ii) relative lack of responses to DDAVP [6] and (iii) insensitivity to oxytocin. These observations suggest that the receptors to vasopressin in blood vessels and liver may resemble each other in their characteristics, but may not resemble the renal receptor to the hormone.

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