Effects of oxytocin upon the endocrine pancreas secretion and glucose turnover in normal man

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Abstract. In normal man oxytocin infusion under basal conditions and at pharmacological doses evoked a rapid surge in plasma glucose and glucagon levels followed by a later increase in plasma insulin levels. Simultaneous [D-³H]glucose infusion indicated that oxytocin also produced a prompt and significant increase in hepatic glucose output with a secondary increase in glucose disappearance rate. Eight healthy volunteers were studied during euglycemic glucose clamp and simultaneous [D-³H]glucose infusion, during suppression of endogenous pancreatic secretion by cyclic somatostatin (250 μg/h) and during exogenous glucagon (67 ng/min) and insulin (0.15 mU · kg⁻¹ · min⁻¹ from 0 to 120 min and 0.40 mU · kg⁻¹ · min⁻¹ from 121 to 240 min) replacement. During the first 60 min oxytocin (0.2 U/min) evoked a transient but significant increase in plasma glucose levels and hepatic glucose output with a simultaneous suppression of the glucose infusion rate. No difference in glucose disappearance and metabolic clearance rates were recorded throughout the clamp irrespective of whether oxytocin was infused or not. So we conclude that oxytocin exerts a hyperglycemic effect through an A-cell stimulation and a glycogenolytic action.

Previous reports have shown that oxytocin affects many metabolic parameters in both animal and man. In particular, Burt et al. (1) showed that oxytocin infusion induced a slight increase in blood glucose levels followed by a secondary hypoglycemic phase. More recently, Dunning et al. (2-3), using islets isolated from rat pancreas, and Vilhardt et al. (4) using conscious dogs, showed that oxytocin acts directly upon the endocrine pancreas. We (5-7) have shown that pharmacological doses of oxytocin affect plasma hormone levels by modulating glucose homeostasis in normal man and diabetic patients.

Nevertheless, no studies have been performed in normal man on the possible oxytocin-induced changes in glucose dynamics, independent of the neurohormone-induced changes in endocrine pancreas secretion. The aim of the present study was to investigate this relationship.

Subjects and Methods
Eight healthy young non-obese (Body Mass Index = 22±1.1, (range 18-25)) men aged 25±3 years (range 22-28) volunteered for the study. None had a family history of diabetes or had taken any drug for at least 3 weeks before starting the experiments. All subjects gave informed consent and the study was approved by the Ethical Committee of our Institution. All were consuming a similar regular, weight-maintaining diet, containing at least 250 g of carbohydrate per day.

Experimental protocol
All subjects were studied in the morning, starting at 08.00-09.00 h after an overnight fast. They were confined to bed and maintained supine throughout each of the experiments. All subjects underwent the following tests: a. dose-response relationship between oxytocin infusion rates and percent increase in plasma glucose levels; b. infusion of...
Blood in After allowed Ci/mmol) c. euglycemic glucose clamp. The tests were carried out in random order, on different days. Oxytocin (Syntocinon®, Sandoz, 450 IU/mg) and an equivalent volume of 0.9% NaCl were administered.

An 18-gauge polyethylene catheter was inserted into an antecubital vein for all infusions. A superficial dorsal hand vein was cannulated in the anterograde fashion with a 19-gauge butterfly needle and kept patent by a slow infusion of 0.9% NaCl; the hand was kept warm by an electric lamp for intermittent sampling of partially arterialized venous blood.

For the dose-response relationship, the different infusion rates were delivered over 60 min and on a different day.

During the clamp study, a contralateral vein was also cannulated for the insertion of the double-lumen catheter to be connected to the Biostator (Life Science Instr, Miles Laboratories, Elkart, USA). At least 1 h was allowed for calibration of the Biostator.

During the euglycemic glucose clamp, oxytocin (0.2 U/min), cyclic somatostatin (250 μg/h, Siltamyl, Italy), and glucagon (3.5 μg/h, Novo Industri A/S, Copenhagen, Denmark) were all delivered from 0 to 240 min by the saline channel of the Biostator. The insulin channel was used to infuse human insulin (Humulin®, Ely-Lilly, Indianapolis, USA) at two different constant rates: 0.15 (from 0 to 120 min) and 0.40 (from 121 to 240 min) mU · kg⁻¹ · min⁻¹ with the aim of performing an euinsulinemic and hyperinsulinemic glucose clamp, respectively. All hormones were dissolved in saline containing 3 g/l human serum albumin (SI, Italy) in order to avoid hormone aggregation (8).

Variable amounts of glucose were infused according to the principles of the euglycemic glucose clamp method as described by De Fronzo et al. (9) and applied to the Biostator by Verdonk et al. (10). The rate of glucose infusion was automatically adjusted by algorithm 7:1 of the Biostator. Glucose was infused as a 20% solution to which 0.26 mmol KCl was added per ml to prevent hypokalemia.

To quantify the rate of glucose appearance and the rate of overall glucose disappearance in the basal state and during glucose clamp we used a primed (20 μCi) continuous (0.2 μCi/min) infusion of [D-³H]glucose (New England Nuclear, Boston, MA; specific activity 11.5 Ci/mmol) dissolved in saline. At least two hours were allowed for isotopic equilibration.

**Blood sampling**

After basal determinations, samples for plasma glucose and hormone levels and glucose turnover were collected every 10 min in the steady-state study and every 20 min in the clamp study.

**Analytical techniques**

With the exception of plasma glucose, which was determined immediately after the end of the experiment by Beckman glucose Auto-Analyzer (Beckman Instruments, Fullerton, CA, intra-assay variability 3.1±0.2%), all other blood samples for hormones were centrifuged after each experiment and plasma was stored at −20°C until assay. Samples for plasma glucose determination were collected in tubes containing a trace of sodium fluoride. Blood samples for C-peptide and immunoreactive glucagon were collected in 10-ml chilled tubes containing 0.6 ml of an EDTA- aprotinin solution (Trasylo®, Bayer, 5 × 10⁶ U/l and Na₂EDTA, 1.2 g/l); blood for insulin determination was collected in heparinized tubes. All tubes were kept in an ice-cold bath throughout the experiments.

Radioimmunoassay methods were used to determine the levels of plasma insulin (Bio-Data kit, Italy, intra-assay variability 3.0±0.4%), glucagon (Byk-Gulden Mat Kit, using Unger’s 30K anti-body, intra-assay variability 4.4±0.6%), and C-peptide (Bio-Gulden Mat Kit, intraassay variability 3.3±0.2%).

Plasma oxytocin levels were determined according to Legros et al. (11). Plasma samples obtained during two experiments of the same series were assayed within the same series in order to eliminate inter-assay variations.

Plasma [D-³H]glucose specific activity was determined as follows: 1-ml aliquots of plasma were deproteinized according to Somogy (12). The resultant filtrate was aliquoted into two 0.5-ml samples that were lyophilized to remove the tritiated water resulting from the metabolism of [D-³H]glucose during the experiment. The dry residue was resuspended with 0.5 ml of distilled water and its radioactivity counted in a refrigerated liquid scintillation counter after addition of 0.5 ml of Aquasol (New England Nuclear). The average radioactivity of each plasma sample was divided by its glucose concentration to obtain the glucose specific activity. Similarly, four aliquots of infused solution of [D-³H]glucose were counted and the average radioactivity as well as the infusion rate were used in the subsequent calculations.

**Calculations and statistical analysis**

The amount of glucose infused necessary to maintain the basal glycemia during insulin infusions (glucose infusion rate) was calculated for each 20-min interval throughout the experiments (expressed in mg · kg⁻¹ · min⁻¹). Rates of glucose turnover (glucose appearance, glucose disappearance, and glucose metabolic clearance rate) were calculated from isotopic dilution data by using the model of Steele (13) and utilizing 20-min integrated values. During the clamp study hepatic glucose production was obtained by the difference between glucose appearance (equal to hepatic glucose output in basal conditions) and the endogenous glucose (or glucose infusion rate).

In the dose-response study, the percent increase in plasma glucose levels was calculated as percent increase, considering the mean plasma glucose calculated during
saline infusion equal to 100% and the highest plasma glucose levels recorded during the 60 min of different oxytocin delivery rates.

All statistical comparisons between two experiments of the same series were performed by the t-test for paired data. A p-value of 0.05 or less was considered of statistical significance. All results are mean plasma concentrations ± SEM.

Results

Dose-response study
Under basal conditions, plasma glucose, insulin and glucagon were similar before oxytocin or saline infusions. The dose-response relationship (Fig. 1) showed that oxytocin delivery promptly rose plasma glucose levels at the rate of 0.1 U/min with a greater increase at 0.2 and 0.3 U/min. No differences in plasma glucose increase were detected between 0.2 and 0.3 U/min. A rate of 0.4 U/min was not used since it produced some adverse reactions in two subjects (see below).

Determination of endocrine pancreatic secretion (Fig. 2) showed that oxytocin infusion (0.2 U/min) produced a significant increase in plasma glucagon levels which changed from 91±11 to 173±37 ng/l (p<0.001) at 20 min and remained significantly elevated until 40 min.

In contrast, plasma insulin levels were only significantly enhanced at the end of the test.

Fig. 1.
Dose-response relationship between oxytocin infusion rate and percent increase in plasma glucose levels. Statistically significant differences vs basal values; *p<0.05; **p<0.01. All results are mean ± SEM (N=8).

Measurements of glucose turnover (Fig. 3) showed that oxytocin infusion (0.2 U/min) caused a prompt, significant increase in hepatic glucose output, whereas the glucose disappearance rate became significantly elevated only in the last 20 min of the test.

Clamp study
As assessed by the similar and progressive decline in plasma C-peptide levels (Fig. 4), endogenous
pancreatic secretion was strongly inhibited by somatostatin infusion irrespective or whether oxytocin was infused or not. Plasma glucagon replacement resulted in stable and physiological peripheral plasma glucagon levels which were close to the basal levels (113±18 vs 118±24 ng/l, NS) and not different at the end of the experiment in both experimental conditions (119±17 vs 124±18 ng/l, NS). During the first 120 min, when insulin was infused at the lowest rate, peripheral plasma insulin levels were similarly clamped at ±71 pmol/l and not different from the respective basal values (73±18 vs 67±25 pmol/l, NS).

From 121 to 240 min, higher insulin infusion rate increased peripheral plasma insulin levels to 358±41 and 362±36 pmol/l (NS) during saline and oxytocin infusion, respectively. Plasma glucagon and C-peptide levels (Fig. 4) remained unchanged.

As compared with saline, infusion of oxytocin evoked a transient but significant surge in plasma glucose levels which peaked at 6.3 mmol/l at 40 min and persisted significantly elevated until 80 min. No difference in plasma glucose levels was observed during the second part of the study.

During the first 60 min of the study oxytocin infusion (0.2 U/min) significantly lowered glucose infusion rate (Fig. 5), but increased hepatic glucose output. However, at the higher insulin infusion rate (0.4 mU·kg⁻¹·min⁻¹), no oxytocin-related effects was observed on glucose homeostasis.

In the presence of comparable peripheral plasma insulin levels, the glucose disappearance rate and glucose metabolic clearance rate were similarly affected by oxytocin or saline delivery throughout the test.

**Plasma oxytocin level variations**
Basal plasma oxytocin levels were ±2.3 mU/l and did not achieve statistically significant differences in any experimental condition before saline and exogenous oxytocin infusion. During exogenous oxytocin infusion of 0.2 U/min, peripheral plasma oxytocin levels rose to 55±13, 121±38 and 204±44 mU/l at 60, 120 and 240 min, respectively.

**Adverse reactions**
Oxytocin infusion (0.2 and 0.3 U/min) caused no adverse reactions in any of the subjects. There was no change in pupil size, blood counts, plasma electrolytes or liver functions tests. Pulse rate (74±9 vs 82±12 beats/min, NS) and mean blood pressure (106±17 vs 93±22 mmHg, NS), also were not significantly affected by oxytocin infusion when infused at the rates described above. By contrast, in the first two patients tested, an oxytocin infusion rate of 0.4 U/min caused headache and tachycardia. The infusion was interrupted and this rate was not studied further.

**Discussion**
Burt et al. (1) first demonstrated that oxytocin infusion evoked a slight increase in blood glucose levels followed by a secondary hypoglycemic phase. A reduction in plasma free fatty acids levels was also observed in young and post-menopausal
women after pharmacological doses of oxytocin; opposite effects were found in young and puerperal women.

Using islets isolated from rat pancreas, Dunning et al. (2) showed that oxytocin stimulates A-cell secretion directly. In particular, these authors showed that both oxytocin and arginine-vasopressin elicited a concentration-dependent stimulation of glucagon release, but failed to influence insulin release in the presence of medium containing 5.6 mmol/l glucose. In contrast, the same authors (5) described a stimulatory effect of oxytocin on both plasma insulin and glucagon levels when an intravenous oxytocin bolus was given in conscious rats.

Altzuler & Hampshire (14) showed that intranasal instillation of oxytocin increased insulin and glucagon secretion in dogs which was confirmed by Vilhardt et al. (4) in their elegant study. They showed the increase in blood glucose levels following oxytocin infusion to be secondary to the glucagon release and oxytocin to exert a direct stimulatory effect on A-cell and possibly B-cell secretions.

Contrasting results have been reported in humans. Chiodera et al. (15) demonstrated a stimulatory effect of oxytocin (6 IU) as a bolus on glucose-induced insulin secretion, whereas Geenen et al. (16) failed to show any effect of a graded oxytocin infusion on carbohydrate metabolism in normal man. More recently, we (5) demonstrated that pharmacological doses of oxytocin evoked a rapid surge in basal plasma glucose and glucagon levels followed by a later increase in plasma insulin levels. In that study, in the absence of glucose turnover data, we hypothesized that oxytocin, at pharmacological doses, has a prevalently hyperglycemic effect by a combined action at the level of the liver (glycogenolytic action) and of the endocrine pancreas (stimulatory agent of A-cell secretion).

Nevertheless, we were not able to show whether oxytocin had only an effect at the level of the liver or also reduced peripheral insulin sensitivity. In the present study we investigated the oxytocin effects on the endocrine pancreas and glucose homeostasis during [D-3H]glucose infusion, which allows us to monitor glucose turnover. Our results confirm that oxytocin has a prevalent stimulatory effect upon A-cell secretion and demonstrate that it enhances hepatic glucose output in basal conditions and during an euglycemic euinsulinemic glucose clamp. By contrast, glucose uptake was only stimulated in the basal condition, but not during the clamp study. In this latter experimental study, hepatic glucose production was sustained by exogenous glucagon infusion and not suppressed by exo-
genous insulin infused to give basal levels. In these conditions the rapid surge in plasma glucose levels and hepatic glucose output can be easily ascribed to a glycogenolytic effect of oxytocin. Such a result is not surprising in the light of the in vitro results of Hems et al. (17) showing oxytocin to have a glyco-
genolytic action on isolated hepatocytes. Nevertheless, this direct hyperglycemic effect of oxytocin could very likely be potentiated by the oxytocin induced A-cell secretion as demonstrated in the steady-state study.

By contrast, the increase in glucose uptake found at the end of the study performed in basal condi-
tions may be due to a secondary increase in plasma insulin levels and not to oxytocin per se. This hypothesis, indeed, seems confirmed by the absence of any effect on peripheral glucose uptake during the clamp study when endogenous glucagon and insulin were replaced at fixed rates by exogenous infusions.

Our glucose kinetic data are in agreement with the results previously reported by Altzuler & Hampshire (18) who showed oxytocin infusion to increase plasma insulin and glucagon levels, as well as glucose appearance and uptake in normal dogs.

The pathophysiological significance of our study is emphasized by the results of Fisher et al. (19,20) showing oxytocin release from the posterior pituitary gland in response to hypoglycemia to be normal in healthy subjects and exaggerated in Type I diabetic patients. Thus, these authors suggested that oxytocin might have a stimulatory or compensatory role in the glucose recovery from hypoglycemia. Moreover, we (7) have recently reported that oxytocin infusion can improve the impaired glucose recovery in subjects with long-standing Type I diabetes in whom the counter-regulatory response is blunted.

As far as the oxytocin effects upon B-cell secretion is concerned, a B-cell response to the rise in plasma glucose levels seems the most suitable explanation.

In conclusion, our results seem to indicate that oxytocin prevalently stimulates A-cell secretion and has a glycogenolytic action, without any effect on peripheral insulin sensitivity.

References

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Fig. 5.
Changes in glucose infusion rate (GIR), hepatic glucose output (HGO), glucose disappearance rate (\(R_d\)) and glucose metabolic clearance rate (MCR) during the eu-
genemic glucose clamp. Oxytocin (0.2 U/min) (○) or saline (●) were infused throughout the experiment. Statistically significant differences: *p<0.05; **p<0.01. All results are mean ± SEM (N=8).

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