Oxytocin Secretion Is Stimulated by Changes in Glucose Metabolism (43788)

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Abstract. **Previous studies have shown that infusion of oxytocin into normal dogs increased plasma levels of insulin and glucagon. These responses were accompanied by increased rates of glucose production and overall glucose uptake. The purpose of the present study was to determine whether, conversely, changes in glucose metabolism would result in changes in oxytocin secretion. In normal dogs, injection of insulin (0.1 U/kg/iv) resulted in increased secretion of oxytocin which coincided with the hypoglycemic nadir, and the oxytocin levels remained elevated for the remaining 60 min, during which time plasma glucose levels were returning to normal. In dogs made diabetic with streptozocin, injection of insulin (1 U/kg/iv) evoked increased oxytocin secretion which began as the plasma glucose levels were falling from the control value of 400 mg/dl to about 170 mg/dl; the oxytocin levels remained above initial values for at least 90 min, during which period plasma glucose returned to normal glycemic values. In normal dogs, infusion of 2-deoxyglucose, which causes intracellular glucopenia, caused a prompt and sustained increase in plasma oxytocin levels. The data suggest that abrupt decreases in availability of glucose in the central nervous system, as induced experimentally by administration of 2-deoxyglucose or by a rapid fall in peripheral glucose levels, evokes secretion of oxytocin.**

[P.S.E.B.M. 1994, Vol 207]

We have previously reported that oxytocin (OT) infusions in normal dogs increasedthe concentration of glucose, insulin and glucagon in the plasma and increased the rates of glucose production and overall glucose uptake by tissues (1). The present study was carried out to determine whether, conversely, alterations in glucose metabolism affect plasma OT levels. The metabolic conditions selected consisted of acutely lowering plasma glucose levels with insulin in normal and diabetic dogs, and by impairing intracellular glucose utilization by administration of 2-deoxyglucose.

Materials and Methods

Experiments were performed on a total of 10 conscious dogs, of either sex, weighing 18–24 kg. Four of these dogs were made diabetic by injection with streptozocin (STZ). The dogs were maintained on a diet of 800 calories/10 kg body wt which consisted of 38% carbohydrate, 39% fat, and 23% protein. The dogs were conditioned to the laboratory setting and were used about 18 hr following ingestion of the daily meal. Each dog was used several times with at least one week between experiments.

Diabetes was induced by injection of STZ, 45 mg/kg, injected iv, at 15 mg/kg on three consecutive days; the drug was dissolved immediately before use in 0.1 *M* sodium citrate at pH 4.3. At least 2 weeks were allowed to elapse following induction of diabetes, and only animals exhibiting plasma glucose levels over 300 mg/dl were used. They were maintained on a mixture of regular and NPH insulin, which was withheld 36 hr prior to the experiment.

Blood samples were obtained through a polyethylene tubing inserted percutaneously into the jugular

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Received December 2, 1993. [P.S.E.B.M. 1994, Vol 207]
Accepted April 14, 1994.

0037-9727/94/2071-0038$10.50/0

vein shortly prior to each experiment. All infusions were into the saphenous vein through a polyethylene tubing, inserted as above, using a multispeed infusion pump (Harvard Apparatus, Boston, MA). Serial blood samples, taken at various intervals as indicated in the figures, were collected in heparinized syringes and transferred immediately to test tubes kept on ice. Following centrifugation, an aliquot of plasma was frozen for glucose analysis by the glucose oxidase technique using the Glucose Analyzer (Beckman).

For determination of OT, 3 ml aliquots of plasma were acidified with 0.6 ml 1 *N* HCl and kept frozen at −20°C until processed and assayed by a highly specific and sensitive radioimmunoassay (2, 3). Briefly, the plasma OT was extracted by passing the aliquot through heat activated Florisil (Sigma Chemical Co., St. Louis, MO) in minicolumns (Isolab, Akron, OH) and eluted with 90% acetone and water. The standard curves were prepared by adding standards (4th International Standard from National Bureau of Standards, London, UK) to charcoal-treated dog plasma samples that were extracted at the same time as the unknowns, thereby correcting for extraction losses. The recovery of added standards was 88.9% ± 8.5% in the range of 5–25 μU/sample or 1.7–8.3 μU/ml. All samples from the same experiment were run in the same assay. The detection limit was 0.25 μU/ml, the intraassay variations were 7%–8% and the interassay variations were 15%–18%. The 4th International Standard from the National Bureau of Standards, UK is determined in units: 1 μU ~ 1.68 pg peptide. The sensitivity and specificity of the antibody used was described in detail earlier (3). The antibody was a gift from Dr. Mariana Morris, Bowman Gray Medical School, Winston Salem, NC. Streptozocin was a gift from Dr. Dulin, Upjohn Co. (Kalamazoo, MI) and 2-deoxyglucose was purchased from Sigma Chemical Co. (St. Louis, MO).

Data analysis and statistical evaluation was carried out as follows. Cochran's C test indicated heteroscadicity of the OT data. Logarithmic transformation, which resulted in homogeneity of variance, was performed before statistical analysis of the data. Significance of the changes in hormone levels was determined by the one-way analysis of variance and multiple range testing (Dunett's test) according to the least squares difference procedure. Means of the two or three pretreatment values in each experiment were used as the initial value. In the figures, results are shown as arithmetic means ± SE. Differences in which $P < 0.05$ were considered to be significant.

Results

Basal Values. Two or three blood samples were taken for oxytocin analysis before any experiment was begun (around 10:00 AM) and these gave a mean plasma value of 2.09 ± 0.21 μU/ml (n = 66) for the normal dogs. Most dogs had relatively stable basal values. The initial levels in the diabetic dogs, measured at 36 hr after the last insulin injection were 1.90 ± 0.40 μU/ml (n = 12) and were not significantly different from levels in normal dogs. The basal levels remained stable during an infusion of 0.9% NaCl of 0.5 ml/min for 90 min (Fig. 1). Although the oxytocin levels tended to be a little higher in the males than in the females, the sampling size was too small to warrant any conclusions.

Effect of Decrease in Plasma Glucose. In order to determine whether oxytocin secretion could be increased in response to a fall in plasma glucose levels, bolus injections of insulin were given intravenously to six normal dogs and four diabetic dogs off insulin. The insulin dose for normal dogs was 0.1 U/kg but was increased to 1 U/kg for the diabetic dogs in order to obtain a significant fall in the plasma glucose. As shown in Figure 2, lowering of plasma glucose by insulin injection in the normal dogs produced a significant rise in oxytocin levels by 30 min, from a basal value of 1.7 ± 0.16 μU/ml to 2.6 + 0.23, increasing to 3.0 + 0.5 by 60 min and the elevated levels persisted during the 60- to 90-min period (mean 3.6 ± 0.59 μU/ml), at which time the plasma glucose levels had returned to near control values. In the diabetic dogs, the rises in oxytocin levels were more marked (Fig. 3). As the plasma glucose levels were falling from control values of about 400 mg/dl to about 170 mg/dl in the first 30 min, the plasma oxytocin levels had already increased significantly to 4.0 ± 1.17 μU/ml ($P < 0.01$) and remained significantly above initial values during the remainder of the 120-min period of observation, reaching a peak value of 5.8 ± 1.6 μU/ml at 90 min ($P < 0.01$). The plasma glucose values fell to about 60 mg/dl toward the end of the observation period.

Effect of Inhibition of Glucose Utilization.

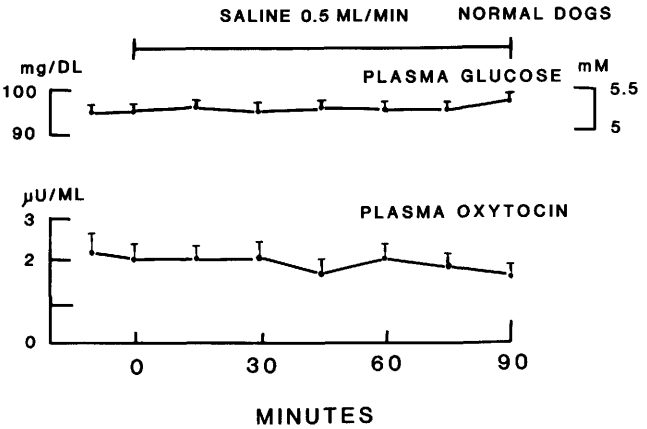

Figure 1. Plasma oxytocin concentrations in resting normal dogs during infusion of physiologic saline (0.5 ml/min) into the saphenous vein. Serial blood samples were taken from an indwelling catheter placed in the jugular vein. Values are means, bars indicate SEM; n = 6. None of the values were significantly different from controls.

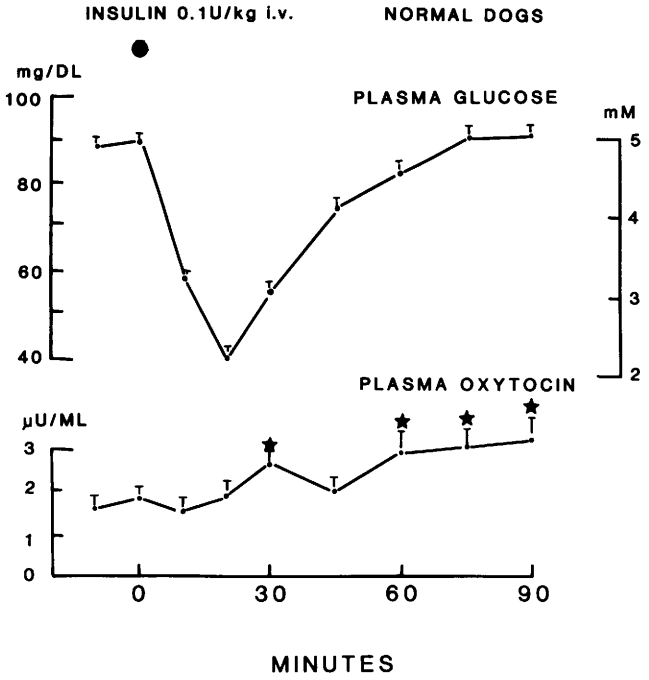

Figure 2. Plasma glucose and oxytocin concentrations in normal dogs given a bolus iv injection of insulin, 0.1 U/kg. Testing for serial randomness of measurements indicated a significant change with time; $n = 6$.

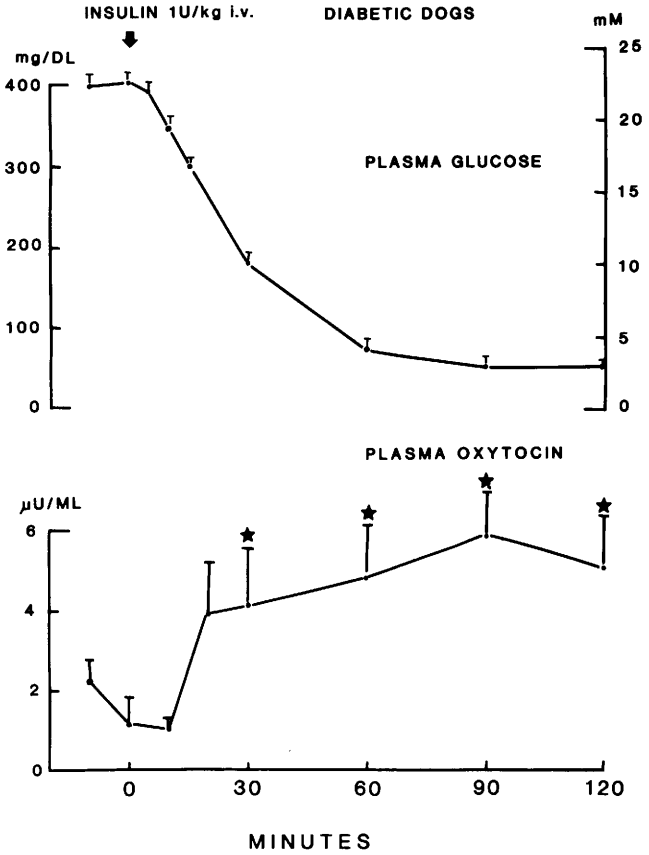

Figure 3. Plasma glucose and oxytocin concentrations in streptozocin-diabetic dogs, 36 hr off insulin, in response to a bolus iv injection of insulin, 1.0 U/kg; $n = 4$. Blood samples taken at intervals of 10 min (0–30 min) and 30 min (30–120 min). The rises in plasma oxytocin levels were statistically significant.

Since the rise in plasma oxytocin levels in the diabetic dogs given a bolus of insulin occurred at a time when plasma glucose values had fallen precipitously but were still well above normal levels, it seemed possible that the increased oxytocin secretion was evoked by a sudden decrease in the availability of glucose to the brain. This was explored by infusing 2-deoxyglucose into normal dogs at 150 mg/kg/30 min, which inhibits glucose uptake (4) and causes intracellular glucopenia (5, 6). As shown in Figure 4, by 30 min of the infusion, the plasma oxytocin levels rose significantly ($P < 0.05$) and remained elevated during the period of observation. Plasma glucose levels also rose during the 2-deoxyglucose infusion, reaching a plateau of 120 ± 3 mg/dl at 45 min, which was maintained during the remainder of the study period. As shown previously, the hyperglycemia was due to a decrease in overall glucose uptake. (4).

Oxytocin Clearance. To ascertain that the rises in plasma oxytocin were due to increased oxytocin secretion rather than decreased clearance caused by glucosuria or hyperglycemia, measurements were made of plasma oxytocin half-life and clearance in three normal dogs infused on separate occasions, either with saline, or with glucose (300 mg/kg/hr) or with phloridzin (0.04 mg/kg/min) which produced a prompt and copius glucosuria. These infusions were for 2 hr, but at 30 min, an oxytocin infusion (500 μU/kg/min) was superimposed to establish plateau levels of oxytocin in the plasma and after 30 min the oxytocin infusion was stopped; blood samples were then collected at 5-min intervals to determine plasma oxytocin half-life. There were no significant differences among the three groups in their plasma OT half lives, which were 13 ± 1, 14 ± 1, and 12 ± 1 min, for saline, glucose and phloridzin, respectively, nor in their metabolic clearance rates, which were 19.7 ± 3.3, 13.4 ± 1.4, and 17.5 ± 0.7 ml/kg/min, respectively ($n = 3$ in each instance). Thus the rises in plasma oxytocin levels observed here were due to increased oxytocin secretion.

Discussion

The present data support the notion that oxytocin secretion can be influenced by changes in glucose metabolism. The nature of the specific stimulus for the oxytocin secretion in these circumstances is unknown, but the present findings offer some possible explanations. The increased oxytocin secretion was evoked in both the normal and diabetic dogs by a rapid fall in the plasma glucose levels brought about by the injection of insulin. In the normal animals, insulin injection produced a marked hypoglycemia, and it might be reasonable to assume that the increased secretion of oxytocin was in response to the hypoglycemia. In man, hypo-

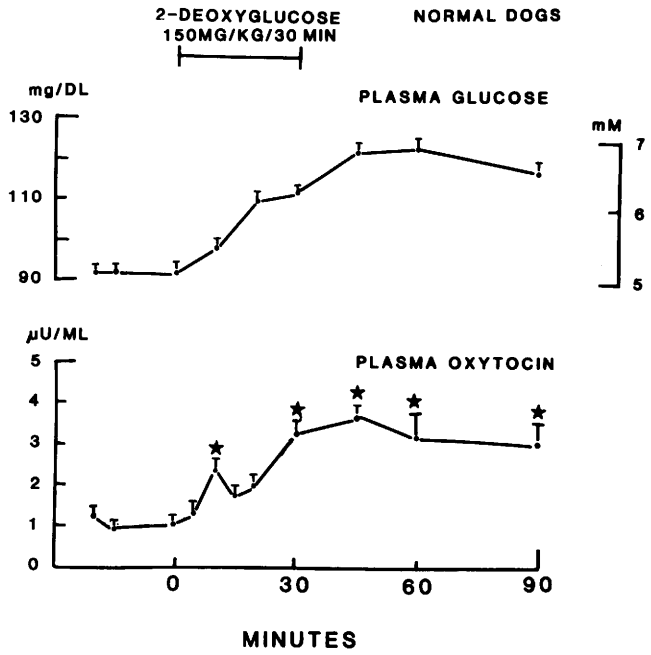

Figure 4. The effect of an iv infusion of 2-deoxy-D-glucose (150 mg/kg/30 min) on plasma glucose and oxytocin concentrations in normal dogs; $n = 6$. Blood samples taken at intervals of 5 min (0–30 min) and 15 min (30–60 min), and at 90 min. The rises in plasma oxytocin levels were statistically significant.

glycemia evoked vasopressin but not oxytocin secretion, except when the opiate antagonist, naloxone, was administered shortly prior to the insulin (7). The latter is in keeping with the finding that endogenous opioids exert an inhibitory tone on oxytocin release (8). The opioid dynorphin coexists and is coreleased with vasopressin from neurosecretory terminals and may exert such an inhibitory effect on oxytocin release; indeed, many stimuli that activate vasopressin release are associated with an inhibitory effect of opioids on oxytocin release (9). In the present study, hypoglycemia was not the stimulus to increase secretion of oxytocin in the diabetic animals, since the increase occurred within 20–30 min following injection of insulin, when plasma glucose levels had fallen from the control values of about 400 mg/dl to about 170 mg/dl and thus were well above normal levels.

A possible stimulus for oxytocin secretion in the above studies might be gleaned from the findings with 2-deoxyglucose infusion. This glucose analogue inhibits glucose uptake (4) and inhibits intracellular glucose metabolism, causing intracellular glucopenia (5, 6). It also results in increased epinephrine secretion (10), but it is unlikely that epinephrine released into the periphery caused the increased secretion of oxytocin since infusion of epinephrine or isoproterenol into normal dogs does not stimulate oxytocin secretion (unpublished results). These studies do not rule out the possibility that 2-deoxyglucose or other as yet unknown stresses may activate the catecholaminergic or serotoninergic neural pathways to the paraventricular nucleus which in turn stimulate the release of oxytocin. In this regard, it has been shown that lowering of glucose in the brain is associated with a rise in hippocampal serotonin and norepinephrine (11), and serotonin stimulates the release of oxytocin into the peripheral circulation when injected into cerebral ventricles (Bardrum and Fuchs, unpublished observations). The initiating signal for oxytocin releases is thus likely to be a lowering of intracellular glucose and increase in serotonin in some brain areas that are involved in modulating oxytocin secretion.

Lowering of glucose in the brain may also explain the increased oxytocin secretion in the diabetic dogs. Although plasma glucose levels following insulin injection did not produce a hypoglycemia, their abrupt fall likely caused a corresponding acute decrement in brain glucose levels which triggered the increased secretion of oxytocin; acute changes in the extracellular brain glucose have been shown to be mirrored by changes in brain glucose (11). Such a stimulus would not be unique for oxytocin. For example, the secretion of growth hormone was shown to be increased not only by hypoglycemia, but also in response to a rapid fall of plasma glucose concentrations from hyperglycemic to control levels (12).

Several additional points regarding the present data warrant comment. Although the rise in oxytocin secretion is evoked by acute changes in plasma glucose levels, the increased oxytocin secretion outlasts the glucose changes and the oxytocin levels are still elevated when the plasma glucose has stabilized (Fig. 3 and 4). This is in agreement with the findings (11) that the increased release of monoamines following stress or lowering of brain glucose was quite slow and that changes in brain glucose are associated with widespread neurochemical changes.

Another possible cause for the increased secretion of oxytocin is that it was due to osmotic changes brought about by the marked fluxes of plasma glucose induced by insulin. This, however, is unlikely, since insulin hypoglycemia was shown not to alter the effective osmolality in normal and insulin-dependent diabetic patients (13). Also, in the present studies, increased secretion of oxytocin was also evident at times when plasma glucose levels were unchanging. It is also unlikely that the diabetic state and withholding insulin for 33–36 hr influenced the oxytocin response, since the diabetic and normal animals had similar plasma oxytocin values in the basal state. Hypovolemia could also be ruled out as a factor. Both normal and diabetic dogs maintained stable oxytocin levels during infusion of saline for similar periods and involving similar frequency of blood sampling. The amount of blood withdrawn in an experiment was about 60 ml, which is about 4% of total body blood and does not represent a hypovolemic stress. Also, between blood sampling,

volume loss was replenished by a saline drip. This does not contradict the finding that increased osmolality produced by infusion of hypertonic saline can stimulate oxytocin as well as vasopressin secretion in the dog (14).

Lastly, since insulin was administered in some of the experiments, the possibility of its direct effect on oxytocin secretion needs to be considered. As noted earlier, infusion of isoproterenol into normal dogs at doses which raised plasma glucose and insulin levels had no effect on oxytocin levels (unpublished results). Furthermore, in the 2-deoxyglucose experiments, there was no rise in plasma insulin whereas oxytocin levels did increase indicating that the specific response is not dependent on increased availability of insulin.

The physiological significance of the effects observed in this study remain to be determined. The changes in glucose levels that evoked increased oxytocin secretion were large and unlikely to occur under normal conditions. However, certain cells in the brain may be sensitive to much smaller changes in intracellular glucose levels than those observed in the peripheral circulation in the present studies and thus may affect oxytocin secretion. The hypothalamic-neurosecretory system has a very high rate of glucose utilization during stimulation (15) and a relative glucopenia in the system may act as a positive feedback to augment secretion of oxytocin as well as that of other hormones. Indeed, in rats made diabetic with streptozocin, there is increased synthesis of oxytocin and vasopressin in the hypothalamus (16). Future studies should provide more direct evidence to substantiate these conjectures.

This work was supported in part by the Warner-Lambert Foundation and by the UPS Foundation. The excellent technical assistance of Dulce Navarro is acknowledged as well. The authors are greatly indebted to Dr. Mariana Morris, Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Winston Salem, NC, for the gift of the oxytocin serum.

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