1 Zipser, R.D., Licht, P., and Bern, H.A., Gen. comp. Endocr. *13* (1969) 382.
2 Etkin, W., and Lehrer, R., Endocrinology *67* (1960) 457.
3 Licht, P., Gen. comp. Endocr. *9* (1967) 49.
4 Meier, A.H., and Farner, D.S., Gen. comp. Endocr. *4* (1964) 584.
5 Nicoll, C.S., in: Handbook of Physiology, vol. 7: Endocrinology, p. 229. American Physiological Society, Washington 1974.
6 May, R.M., C. r. Soc. exp. Biol. *124* (1937) 920.
7 Goldberg, R.C., and Knobil, E., Endocrinology *61* (1957) 742.
8 Hertz, R., Endocrinology *65* (1959) 926.
9 Meites, J., Nicoll, C.S., and Talwalker, P.K., in: Advances in Neurobiology, p. 238. Ed. A.V. Nalbandov. Un. Illinois Press, Urbana 1963.
10 Welsch, C.W., Negro-Vilar, A., and Meites, J., Neuroendocrinology *3* (1968) 238.
11 Adler, R.A., Herzberg, V.L., and Sokol, H.W., Life Sci. *32* (1983) 2957.
12 Schalch, D.S., and Reichlin, S., Endocrinology *79* (1966) 275.
13 Panerai, A.E., Gil-Ad, I., Cocchi, D., Locatelli, V., Rossi, G.L., and Müller, E.E., J. Endocr. *72* (1977) 301.
14 Witorsch, R.J., and Kitay, J.I., Endocrinology *91* (1972) 764.
15 Marshall, S., Huang, H.H., Kledzik, G.S., Campbell, G.A., and Meites, J., Endocrinology *102* (1978) 869.
16 Murphy, B.E.P., J. clin. Endocr. Metab. *27* (1967) 973.
17 Udeschini, G., Cocchi, D., Panerai, A.E., Gil-Ad, I., Rossi, G.L., Chiodini, P.G., Liuzzi, A., and Müller, E.E., Endocrinology *98* (1976) 807.
18 Chen, C.L., Amenomori, Y., Lu, H.K., Vogt, J.L., and Meites, J., Neuroendocrinology *94* (1974) 1709.
19 MacLeod, R.M., Smith, M.C., and Dewitt, G.W., Endocrinology *79* (1966) 1149.
20 Fang, V.S., Refetoff, S., and Rosenfield, R.S., Endocrinology *95* (1974) 991.

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Antidiuretic effects of oxytocin in the Brattleboro rat

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Summary. The antidiuretic activity of oxytocin (OT) was measured in Brattleboro rats with congenital diabetes insipidus. A dose dependent antidiuretic response was found in animals receiving chronic infusions of 0.1 μg/h, 1.0 μg/h, and 5 μg/h of OT. OT infused at the rate of 5 μg/h over a 7-day period completely reversed the symptoms of diabetes insipidus. The results support the concept that OT serves as a weak agonist of vasopressin at the level of the kidney and at pharmacological levels exhibits antidiuretic activity.
Key words. Oxytocin; antidiuretic activity; Brattleboro rats.

Experiments on the renal effects of oxytocin (OT) have yielded variable results, but most studies indicate that OT serves as both a weak agonist at the renal vasopressin receptor and has important natriuretic effects[1–5]. Elevated levels of OT in the plasma and decreased stores of OT in the pituitary are found in the vasopressin-deficient Brattleboro rat[6,7] indicating that OT is being secreted in response to the severe fluid and electrolyte imbalances resulting from the absence of vasopressin and raising the possibility that OT might correct some of the fluid inbalance. Edwards, LaRochelle, and Gallai [3] found that plasma OT levels increased in the Brattleboro rat concomitant with increased urine osmolalities during a 24-h dehydration study. However, in a second part of their study they suggested that OT had little or no role in the increased concentrating ability of dehydrated DI rats because infusions to produce similar elevated levels of OT in normally hydrated Brattleboro rats led to only a slight rise in urine osmolality.

The present study was undertaken to further clarify the role of OT in fluid regulation in the DI rat. Our research group is investigating the structural and functional development of neural transplants using the Brattleboro rat as a model [8,9]. These studies entail grafting fetal vasopressin neurons into the third ventricle of Brattleboro hosts and measuring the ability of the transplants to ameliorate the host's diabetes insipidus. The proper evaluation of our experiments requires a better understanding of the effects of OT since the transplants may stimulate (or inhibit) OT release. Furthermore, as OT levels may be permanently increased following transplantation, previous results with acute infusions of OT might not be applicable to our studies. Therefore, in the present set of experiments, OT was chronically infused via osmotic minipumps to better mimic the effects of constant elevated levels of OT which may result from transplantation.

Materials and methods. Adult male Brattleboro rats (Blue Spruce Farms, Altamont, NY) homozygous for the diabetes insipidus trait and weighing 225–450 g were used. They had been castrated more than twenty days prior to the beginning of this experiment as part of preliminary study on the effects of sex steroids on water balance; the castration caused only very minor changes, and all animals began the present study from a common baseline. The rats were maintained in stainless steel metabolism cages on a 12/12-h light-dark cycle with food and water provided ad libitum. They were anesthetized with ether and an Alzet osmotic minipump (Alza, Palo Alto, CA), calibrated to deliver 1.0 μl/h of solution, was implanted subcutaneously above the scapula. Pumps were filled either with physiological saline or solutions of synthetic OT (Bachem Inc., Torrance, CA) in saline at titers of 0.1 μg/μl, 1.0 μg/μl, or 5.0 μg/μl. Water consumption and urine osmolality were monitored daily in all animals for a minimum of three days prior to and three days after pump implantation. The animals receiving 5 μg/h OT were monitored for seven days after implantation.

Animals receiving saline, 0.1 μg/h and 1.0 μg/h OT, were used to

Oxytocin measurements

Test Group	Plasma oxytocin μU/ml	Hypothalamic oxytocin mU/total	Pituitary oxytocin mU/total	Urine osmolality mOsm/L
Saline (n = 8)	10.7 ± 2.7	7.51 ± 0.82	749 ± 62	250 ± 20
0.1 μg (n = 6)	71.4 ± 13.4*	8.75 ± 0.42	1653 ± 571	343 ± 47
1.0 μg/h (n = 8)	227.3 ± 21*	8.83 ± 1.4	729 ± 140	853 ± 35*

Data are presented as mean values ± SEM. *Significantly different for the saline controls $p < 0.005$. 1 μU OT = 1.65 ± 0.12 pg synthetic OT (Bachem).

determine plasma levels resulting from chronic infusions of oxytocin and the correlation between plasma OT and OT concentrations in the hypothalamus and pituitary. These rats were decapitated on the third day following pump implantation and trunk blood was collected in test tubes containing 200 µl of heparin. Blood samples were kept on ice until they were centrifuged and the plasma removed and frozen. The entire anterior hypothalamus including the median eminence and paraventricular and supraoptic nuclei was dissected out and homogenized. The posterior pituitary was separately homogenized. The tissue samples were homogenized in 1 ml of 0.1 N HCl in a glass homogenizer, the homogenizer was rinsed once with 1 ml of 0.1 N HCl. Rinse and homogenate solutions were combined, centrifuged at 1000 × g and the resulting supernatant collected and frozen. Ten µl of fluid recovered from each osmopump was diluted to 1 ml in 0.1 N HCl and frozen.

Samples were assayed for OT content using procedures described in detail elsewhere[10]. The standard curve was prepared with USP pituitary reference standard (US Pharmaceuticals, Rockville, MD) which gave identical displacement with synthetic OT. Intraassay coefficients of variation were from 1.4 to 4.4% for various points of the standard curve and the interassay coefficient of variation from 8.3 to 15%. Plasma samples were extracted by precipitation of protein with 2 ml acetone and delipidation of the supernatant with 2 ml anhydrous ether. The acetone phase was dried and reconstituted in 500 µl of 0.01 M KPO_4 buffer with added NaCl (8.7 gm/l; pH 7.4). Recovery of synthetic OT added to OT-free plasma was 75% ± 2 SEM. The tissue homogenates were run for assay after adjusting supernatant pH to 7.4.

All data were statistically evaluated by analysis of variance.

Results. The results of the present study demonstrate a dose dependent antidiuretic activity for OT in animals receiving chronic infusions (fig. 1, A and B) and a capability, at high titers, for OT to completely reverse the symptoms of diabetes insipidus in Brattleboro rats (fig. 2).

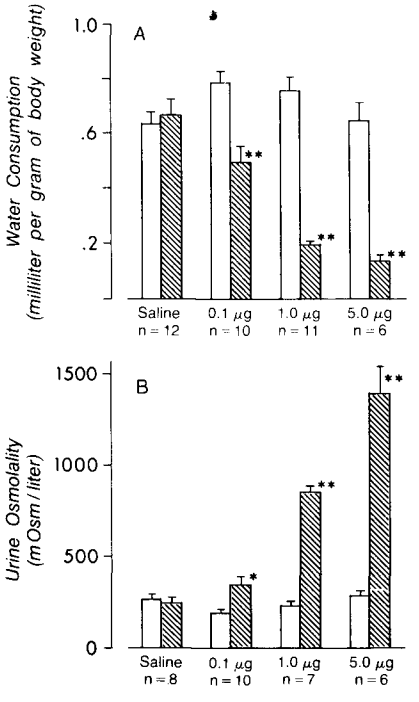

Figure 1. Changes in water consumption (A) and urine osmolality (B) are shown following oxytocin administration. The three days averages prior to osmopump implantation (clear columns) are compared to measurement on the third day after implantation (hatched columns). Bars indicate the SE of the mean. *p < 0.01; **p < 0.001.

Brattleboro rats receiving saline via the osmopumps had plasma levels of OT ranging from 9.5 to 24.6 µU/ml (see table). The chronic subcutaneous infusion of 0.1 µg OT/h resulted in circulating levels of OT averaging 71.4 µU/ml and infusions of 1.0 µg/h led to average levels of 227 µU/ml. Hypothalamic OT levels were not significantly affected by the increases in circulating levels of OT. Posterior pituitary storage levels of OT showed an increased variance with the 0.1 µg/h rate of infusion, but otherwise were not significantly different from saline or 1.0 µg/h OT recipients.

Discussion. OT, infused over a 7-day period, altered water metabolism in a pattern very similar to that observed with vasopressin administration[10]. As with similar studies employing chronic vasopressin injections or infusions, the maximal response as measured by urine osmolality was not seen until after at least four to six days of treatment[10]. Valtin[6] has suggested that the impaired response to vasopressin is due to the time period required for sufficient concentration gradients to be established in the renal convoluted tubules and collecting ducts. DI medullary osmolality is initially low and as it gradually increases under vasopressin stimulation, the ability of the kidney to concentrate urine increases. The parallel effects of vasopressin and OT on the kidney suggests (but does not prove) that both hormones employ similar mechanisms of action. That is, both may, through receptor mediated action, cause the distal convoluted tubules and collecting ducts to become permeable to water. As indicated by the older literature (for a comprehensive review, see Peters and Roch-Ramel[5]), this antiduretic effect of OT can be separated from its natriuretic properties.

The results of the present study support the concept that OT serves as a weak agonist of vasopressin at the level of the kidney. Three to four days of infusion of OT was necessary to achieve maximum antiduiresis. However, a significant decrease in urine output was not noted until plasma OT was above 70 µU/ml, and urine osmolality did not become markedly concentrated until plasma OT was above 200 µU/ml. As the level of OT in the untreated Brattleboro rat was less than 20 µU/ml, it appears unlikely that secreted OT will ameliorate the diabetes insipidus. We also documented that chronic levels of OT in the plasma of greater than 200 µU/ml does not effect the content of OT in the hypothalamus.

Thus, while chronic OT infusions in the Brattleboro DI rat may result in greater antidiuresis than acute injections, the titers of OT in the plasma needed to significantly alter the fluid balance are still several times greater than physiological levels in these

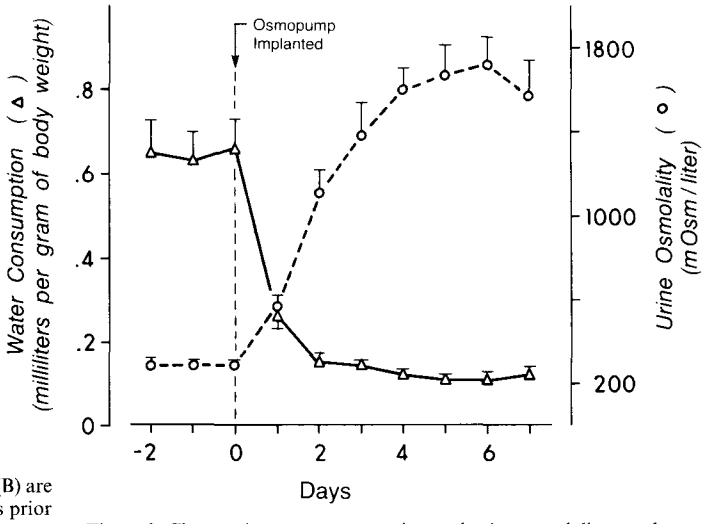

Figure 2. Changes in water consumption and urine osmolality are shown for gonadectomized male Brattleboro rats (n = 6) receiving chronic infusions of 5 µg oxytocin/h.

same rats. Chronic infusions of OT produce the same conclusions as short-term infusions; that intrinsic OT does not alter the fluid balance in the DI rat.

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1 Sawyer, W. H., and Valtin, H., Endocrinology *80* (1967) 207.
2 Gellai, M., LaRochelle, F. T., and Valtin, H., in: Hormonal Regulation by Sodium Excretion, p. 121. Eds B. Lichardus, R. W. Schrier and J. Ponec. Elsevier/North Holland Biomedical Press, Amsterdam 1980.
3 Edwards, B. R., LaRochelle, F. T. Jr, and Gellai, M., Ann. N.Y. Acad. Sci. *394* (1982) 497.
4 Forsling, M. L., Brimble, M. J., and Balment, R. J., Acta endocr. *100* (1982) 216.
5 Peters, G., and Roch-Ramel, F., Int. Ency. Pharm. Ther., section 41, vol. 1 (1970) 229.
6 Valtin, H., Am. J. Med. *42* (1967) 814.
7 Balment, R. J., Brimble, M. J., and Forsling, M. L., Ann. N.Y. Acad. Sci. *394* (1982) 241.
8 Gash, D. M., Sladek, C. D., and Sladek, J. R. Jr, Peptides *1*, suppl. 1 (1980) 125.
9 Sladek, J. R. Jr, and Gash, D. M., in: Neural transplants: Development and Function, p. 243. Eds J. R. Sladek, Jr, and D. M. Gash. Plenum, New York 1984.
10 Amico, J. A., Seif, S. M., and Robinson, A. G., J. clin. Endocr. Metab. *52* (1981) 988.
11 Gash, D. M., Warren, P. H., Dick, L. B., Sladek, J. R. Jr, and Ison, J. R., Ann. N.Y. Acad. Sci. *394* (1982) 672.
12 Robinson, I. C. A. F., Clark, R. G., Fairhall, K. M., Jones, P. M., and Parsons, J. A., Ann. N.Y. Acad. Sci. *394* (1982) 285.

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Effects of PGE_2 and $PGF_{2\alpha}$ on the simulation by noradrenaline and oxytocin of human cervical muscle activity at term

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Summary. Cervical specimens were obtained by needle biopsy in connection with caesarean section at term pregnancy. The preparations were superfused in an organ chamber and contractions were registered isometrically. Prostaglandin (PG) E_2 and $F_{2\alpha}$ inhibited spontaneous contractions. The stimulatory action of noradrenaline was not influenced by $PGF_{2\alpha}$ but was reduced by PGE_2 whereas both PGs abolished the excitatory effect of oxytocin.
Key words. Prostaglandins; uterus; cervix; pregnancy; smooth muscle; catecholamines; oxytocin.

Clinical and experimental studies have documented the importance of prostaglandins (PGs) as regulators of contractile activity in the myometrium of the human uterine body[1]. With respect to the human cervix the interest has been mainly focused on the influence of PGs on 'ripening' processes in the cervical connective tissue. In a recent study, however, we investigated the influence of various natural PGs on cervical smooth muscle contractility in nonpregnant and early pregnant women[2,3]. PGE_2, PGI_2 and 6-keto-$PGF_{1\alpha}$ were all shown to inhibit muscle activity whereas $PGF_{2\alpha}$ did not affect contractility at all. The inability of $PGF_{2\alpha}$ to influence cervical musculature was an unexpected finding, since $PGF_{2\alpha}$ is known as a potent stimulator of smooth muscle not only within the reproductive tract but also in other organ systems[1,4]. The present work, conducted on cervical specimens obtained at term pregnancy, concerns the influence of PGE_2 and $PGF_{2\alpha}$ on contractile activity in relation to the action of noradrenaline and oxytocin.

Material and methods. Cervical tissue was obtained from 20 women undergoing elective caesarean section in the 38–40th week of pregnancy. The tissue was isolated by the use of a biopsy needle (Tru Cut, Travenol, Deersfield, Ill., USA), inserted via the transverse incision in the lower uterine segment, using one finger to identify the internal os. The specimens (approx. 1.5 × 15 mm) were placed in ice-chilled Krebs-Ringer bicarbonate (KRB) or HEPES (Sigma Chemical Co., St Louis, Miss., USA) buffer (pH approx. 7.38) and immediately transferred to the laboratory. Within 30 min muscle strips with a length of 4–5 mm were mounted in a tissue chamber superfused by KRB or HEPES buffer fortified with 10 mM glucose under continuous oxygenation (5% CO_2 in O_2 or pure O_2). One end of the strip was connected to a force transducer (Grass model FTO3) and contractile activity was registered isometrically under a passive tension of 5 mN. $PGF_{2\alpha}$ (Prostin®, Upjohn Co., Kalamazoo, Mi., USA), oxytocin (Syntocinon®, Sandoz AG, Basel, Switzerland) and noradrenaline (ACO Ltd, Sweden) were injected as minute volumes into the system via a plastic valve connected to the superfusion tubing[3].

Results. During the present series of experiments two different buffer systems were used. Neither the spontaneous muscle activity, nor the response to an added drug was dependent on the buffer used. $PGF_{2\alpha}$ (10^{-7}–10^{-6} g/ml, n = 6) and PGE_2 (10^{-10}–10^{-7} g/ml, n = 6) induced a total inhibition of spontaneous contractions. Noradrenaline (10^{-7}–10^{-6} M, n = 14), and oxytocin (10–100 mU/ml, n = 8), stimulated the contractile activity. PGE_2 (10^{-7} g/ml, n = 7) administered before or after administration of noradrenaline considerably reduced this excitatory response (fig.). $PGF_{2\alpha}$ (10^{-6} g/ml, n = 9), on the other hand, had no significant effect on the response to noradrenaline. The stimulatory effect of oxytocin, which was less powerful than that of noradrenaline, was totally abolished by PGE_2 (10^{-7} g/ml, n = 3) and also by $PGF_{2\alpha}$ (10^{-6} g/ml, n = 3).

Discussion. The present results document the fact that the musculature of the uterine cervix differs functionally from the myometrium of the uterine body. Thus, $PGF_{2\alpha}$ inhibits cervical muscle activity but stimulates contractions of the uterine fundus and has little effect on the lower uterine segment[5] during labor. This segmentally differentiated effect of $PGF_{2\alpha}$ appears to be highly appropriate from a biodynamic point of view with respect to parturition, although the mechanisms which underlie the divergent response to $PGF_{2\alpha}$ are unknown. The potency of $PGF_{2\alpha}$ as an inhibitor of cervical muscle activity was, however, considerably lower than that of PGE_2. The high sensitivity of cervical tissue to PGE_2 is interesting in view of the effectiveness of PGE_2 as a primer for cervical ripening[6]. This process, usually regarded as involving only the cervical connective tissue, may also involve the smooth muscle elements in the cervix. Accordingly, both