Oxytocin Induces Maternal Behavior in Virgin Female Rats

Abstract. *Intracerebroventricular administration of oxytocin to virgin female rats that had been ovariectomized and primed with estrogen 48 hours previously induced a rapid onset of full maternal behavior. The maternal behavior persisted and its incidence was dose-related. Tocinoic acid, the ring structure of oxytocin, also rapidly induced the onset of persistent, full maternal behavior. Arginine vasopressin induced persistent maternal behavior, but this behavior had a later onset. Prostaglandin $F_{2\alpha}$ induced strong partial maternal behavior, which showed early onset but did not persist. Many other peptides, ovarian steroids, and prostaglandin E_2 were no more effective than saline. These findings suggest that the release of oxytocin and prostaglandin $F_{2\alpha}$ during labor may promote maternal behavior in rats.*

Oxytocin is an oligopeptide consisting of a ring structure of five and a side chain of three amino acids (*1*). It is synthesized in cell bodies of the paraventricular and supraoptic nuclei of the hypothalamus and transported through axons to the posterior pituitary gland (*2*) from which it is secreted during parturition (*3*) and nursing (*4*). Oxytocin given intracerebroventricularly (ICV) to virgin female rats caused a rapid onset of a full spectrum of maternal behavior, provided the estrogen levels were elevated (*5*). We now report that this effect of oxytocin is dose-dependent, that it is reasonably specific, and that exceptions to specificity can be understood either as functions of molecular similarities or as expressions of known physiological relationships. Active substances differ not only in potency but also in rapidity of onset and duration of effect.

For these experiments we used virgin Sprague-Dawley female rats (250 to 350 g; Zivic Miller Laboratories) in each of which we had implanted a cannula (Plastic Products Company) stereotaxically in the left lateral brain ventricle (*5*). One week later the rats were ovariectomized and injected subcutaneously with estradiol benzoate (100 μg/kg) in corn oil. Forty-six hours later the animals were placed in individual observation cages (44 cm long, 21 cm wide, and 20 cm high) containing 20 pieces of paper toweling (6 by 6 cm) for nesting material. After 2 hours of habituation the animals were removed briefly from their cages and given ICV injections of test substances dissolved or sonicated in 0.9 percent saline, *p*H 5.0. Each animal received 10 μl of a test solution or saline delivered in 50 seconds by means of a Hamilton syringe connected by polyethylene tubing to the lateral ventricular cannula.

While each adult rat was being injected, three rat pups (2 to 8 days old) were placed at the apices of an imaginary triangle, 10 cm on a side, centered in the observation cage. The pieces of paper toweling were evenly redistributed. Each adult animal was returned to the center of its cage immediately after the ICV injection was completed. We studied 10 to 14 animals at each session. Two or more sessions were devoted to the study of each dose of oxytocin and of each of the other substances tested. Each animal was used only once.

For 2 hours after the rats received an injection an observer (who did not know which substance was being tested) recorded the responses of each rat toward the foster pups (*6*). Later we added a third hour of observation (from hours 4 to 5 after injection) because we noted that the onset of maternal behavior induced by arginine vasopressin (AVP) was often somewhat delayed. Five categories of maternal behavior were scored: grouping and regrouping of pups; licking of pups; crouching over grouped pups; nest building; and retrieval of pups. At the end of each hour each animal was assigned a behavioral score based on one point for each category in which criteria were met during that hour. The five categories of maternal behavior and the criteria for each have been described elsewhere (*5*). For expressions of the intensity of maternal behavior we established the category of full maternal behavior (FMB), animals achieving a score of 5, and the category of strong partial maternal behavior (SPMB), animals

Table 1. The percentage of animals in each treatment group (*N*) displaying full maternal behavior from 0 to 60 minutes, 60 to 120 minutes, or 240 to 300 minutes after receiving either no injection or an ICV injection of one of the following: 400 ng of oxytocin, a dose of a test substance equimolar to 400 ng of oxytocin, or saline alone. Substances are ranked, from top to bottom, in order of decreasing incidence of full maternal behavior during the first hour of observation. The animals were ovariectomized and primed with estrogen 48 hours before they were given the ICV injections in order to standardize the concentrations of estrogen in the plasma of the animals. Although estrogen priming after ovariectomy may have produced variable titers of estrogen in individual animals after 48 hours and, therefore, variable sensitivities to the test substances, this would not have biased the results toward one or another test substance because of the number of animals we used to test each substance. The sources of the test substances were as follows: V. Hruby, oxytocin, arginine vasopressin, lysine vasopressin, tocinoic acid, and Pro-Leu-Gly-NH_2; C. H. Li, human β-endorphin; Bachem, Inc., oxytocin (lot 9721), arginine vasopressin (lot R2383), lysine vasopressin (lot R2644), arginine vasotocin (lot R1750), luteinizing hormone–releasing hormone diacetate-4H_2O (lot R1164), neurotensin (lot 6529), pressinoic acid (lot R2132), and bradykinin (lot R3966); Sigma, progesterone (lot 100F-0086), 17β-estradiol (lot 19C-0519), prolactin (lot 38C-0094), prostaglandin $F_{2\alpha}$ (lot 129C-0358), and prostaglandin E_2 (lot 807-4025); Abbott, thyrotropin-releasing hormone (lot 38-430-AL); and Beckman, substance P (lot A0931). At the very low concentrations required all substances were soluble in water except estrogen and progesterone. These two substances were sonicated for 30 minutes in normal saline before they were injected; nevertheless, we cannot be sure that doses equimolar to 400 ng of oxytocin were delivered.

Substance	*N*	Percentage of animals fully maternal		
		0 to 60 minutes	60 to 120 minutes	240 to 300 minutes
Oxytocin	107	72*	72*	72* (*N* = 58)†
Tocinoic acid	24	50‡	50‡	54‡
β-Endorphin	11	27	27	
Luteinizing hormone–releasing hormone	23	26	26	
Thyrotropin-releasing hormone	12	25	25	
Pro-Leu-Gly-NH_2	12	25	25	
Prolactin	12	25	25	
17β-Estradiol	12	25	25	
Progesterone	12	25	25	
Prostaglandin E_2	14	21	21	36
Lysine vasopressin	29	21	24	24
Pressinoic acid	15	20	20	20
Bradykinin	10	20	20	
Arginine vasotocin	11	18	27	
Saline	51	18	18	19 (*N* = 26)†
Prostaglandin $F_{2\alpha}$	19	16	16	21
Arginine vasopressin	31	16	42§	55‡
No ICV injection	20	15	15	15
Substance P	17	12	12	
Neurotensin	11	9	9	

*$P < .001$. †*N* shows the number of animals observed during the third observation period added part way through the experiment. ‡$P < .01$. §$P < .05$ compared to the group treated with saline (Fisher's exact probability test).

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achieving a score of 3 or 4 (but not 5). Seven percent of all the animals we studied, regardless of the injection they received, killed one or more pups. Any animal that killed a pup was removed and given a behavioral score of "kill" (*6*).

The doses of oxytocin (lot No. 9721, Bachem) ranged from 100 to 400 ng. Results pertaining to the first hour after ICV injection are shown in Fig. 1. The incidence of FMB was linearly related to the dose of oxytocin ($P < .01$). As observed previously (*5*), oxytocin usually induced onset of FMB within 1 hour.

We also explored the specificity of the oxytocin effect, noting the frequency, intensity, rapidity of onset, and duration of maternal behavior induced by the drug. Animals received doses of test substances equimolar to 400 ng of oxytocin (for positive comparison), saline, or no ICV injection. At each session at least two animals received oxytocin; one or two received saline. We used oxytocin from lot No. 9721 or a lot synthesized by V. Hruby; the results obtained with the two lots were almost identical and were therefore combined.

The incidence of FMB in animals injected with oxytocin or tocinoic acid was statistically significant during all time periods (Table 1). Arginine vasopressin produced a significant incidence of FMB during the second and third but not the first time period. No other substance showed so late an onset of FMB or SPMB. Of the animals receiving saline or a substance other than AVP, 140 showed FMB during the first hour after injection. Only six animals showed an onset of FMB at a later time. Just as the onset of FMB tended to be early so did it tend to persist: only three animals that showed FMB during the first hour did not show it at later hours.

The maternal behavior also tended either to be complete or virtually absent. For this reason analyses based on distribution of scores yielded no more information than analyses of the incidence of FMB. One substance, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), was exceptional in this regard. Of 19 animals injected with $PGF_{2\alpha}$ ten showed SPMB during the first hour ($P < .002$ compared to controls injected with saline). This effect did not persist; nine of the ten animals that showed SPMB during the first hour did not show it during the final hour of observation (data not shown).

These results support our previous findings that ICV injections of oxytocin rapidly induce a high incidence of FMB in ovariectomized, estrogen-primed virgin rats. The efficacy of tocinoic acid (the ring structure of oxytocin) and the impotence of Pro-Leu-Gly-NH_2 (the side chain structure of oxytocin) suggest that it is the ring structure of the oxytocin molecule that interacts with putative brain receptors that mediate the onset of maternal behavior. A dose of 400 ng of oxytocin is far in excess of the quantity measured in cerebrospinal fluid under physiological conditions (*7*). This dose may be required, however, to penetrate to behavior-mediating sites that may be remote from the brain ventricles.

The necessity for the presence of adequate estrogen for the action of oxytocin was reported previously (*5*). Estrogen administration may facilitate oxytocin-induced maternal behavior by increasing oxytocin receptor availability. The low incidence of FMB in ovariectomized, estrogen-primed animals that received saline or no ICV injection may result from estrogen sensitization to endogenous oxytocin or from a direct effect of the administered estrogen.

Like oxytocin, arginine vasopressin, which also occurs in the posterior pituitary of the rat (*8*), is released during labor in some species (*9*). The amino acid sequence of the two octapeptides differ in only two positions (*1*). At appropriate dosages both peptides produce uterine contraction (*10*), milk ejection (*11*), antidiuresis (*12*), and retention of avoidance behavior (*13*). Induction of maternal behavior appears to be another shared effect. However, the difference in latency of onset of maternal behavior after oxytocin and AVP administration suggests that these peptides may exert their effects through different mechanisms. In our previous work, AVP was ineffective in intact estrous cycling virgin rats in inducing FMB within 2 hours, suggesting that the AVP effect is more dependent on estrogen than is the effect of oxytocin.

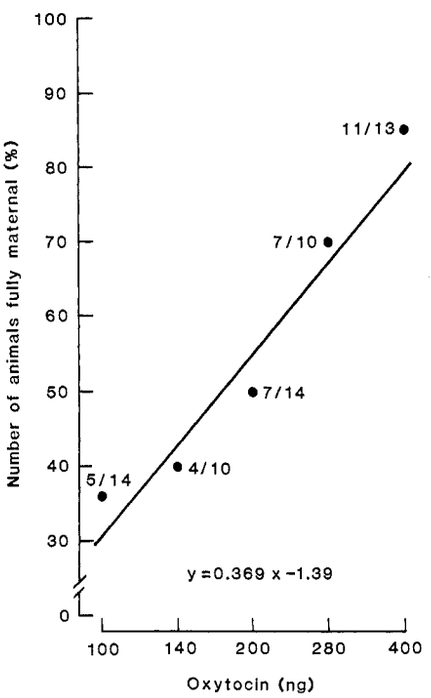

Fig. 1. Percentage of virgin female rats displaying full maternal behavior within 1 hour after receiving an intracerebroventricular injection of oxytocin (between 100 and 400 ng) given 48 hours after ovariectomy and priming with estrogen (100 μg of estradiol benzoate per kilogram of body weight). Eighteen percent of rats (3 out of 17) receiving saline displayed full maternal behavior. For the horizontal axis we use a log natural scale, with the numbers showing the doses of oxytocin administered.

The observation that $PGF_{2\alpha}$ induces SPMB is interesting in view of recently reported interactions between prostaglandins and oxytocin. Oxytocin can enhance $PGF_{2\alpha}$ release from the endometrium of estrogen-primed animals (*14*). Intracerebroventricular administration of $PGF_{2\alpha}$ to lactating rats selectively increases the firing rate of oxytocin-containing neurons of the paraventricular nucleus for 20 to 30 minutes after injection (*15*). Thus, a mutually reinforcing interaction between $PGF_{2\alpha}$ and oxytocin may occur in both the uterus and brain of parturient rats. The former may enhance the expulsion of the fetus; the latter may trigger the onset of maternal response toward offspring.

Recent immunohistochemical studies (*16*) indicate that oxytocin-containing (and AVP-containing) pathways are widely distributed to extrahypothalamic areas of the brain. The present results suggest that oxytocin released within the brain may contribute to the rapid onset of postpartum maternal behavior.

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6. For each observation hour the data were considered in two ways: (i) the effect of a test substance, compared to saline, on the distribution of maternal behavior scores (kill, 0, 1, 2, 3, 4, and 5) and (ii) the effect of a test substance on the incidence of defined intensities of maternal behavior. For statistical comparisons of the dis-

tribution of scores we used the Wilcoxon test with adjustment for ties, one-tailed interpretation. Several findings emerged at high levels of confidence. However, the same findings emerged from the simpler technique of comparing the proportion of animals in treatment groups (versus saline) that met criteria for FMB or SPMB.

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17. We thank V. Hruby and C. H. Li for providing peptides, D. Quade and C. Chase for statistical assistance, and D. Yarbrough for manuscript preparation. Supported by NIMH grant MH-32316, ADAMHA grant MH-34933, and a career scientist award to A.J.P. (MH-22536).

24 February 1982

Dolphins and the Bends

Ridgway and Howard (*1*) conclude that "the mechanism by which dolphins avoid decompression sickness on dive schedules known to produce the syndrome in man is not yet completely understood." The formation of bubbles that cause the problems of decompression sickness probably depends on the existence of tiny gas nuclei on which the bubbles form. A single brief excursion to a depth of 200 m reduces bends in rats during a subsequent dive (*2*), and ultrasonic observations suggest that repeated excursions to lesser depths could also reduce the incidence of bends by forcing minute bubble nuclei back into solution. Observations of decompressed samples of gelatin suggest how such changes might come about (*3*). Thus the periodic swimming up and down of dolphins could effectively crush out bubble nuclei.

There is considerable interest in the mechanism of any nonthermal effect of high-frequency sound waves on the body, such as the effect on eye pressure and glaucoma (*4*), especially because of the increasing use of medical ultrasonic equipment. If stable, tiny bubbles exist in normal animal tissue—and the difference in the effects of decompression on whales and men suggest that they do—then in a man exposed to high-frequency sound these bubbles would vibrate and could cause tissue alteration (*5*).

The difference between whales and humans may not be large since one analysis of data from human dives (*6*) suggests that a dive to 100 m allows a safe pressure reduction of 2 to 3 atm, which approximates the conditions described in (*1*). The suggestion that whales must avoid the bends while other creatures do not may not be strictly true. Although most fish do not rapidly cycle between the surface and feeding depths as dolphins do, we have observed goldfish to be relatively inert to decompression death when many bubbles are present (*7*), but marine mammals may sometimes suffer decompression sickness (*8*).

Even if dolphins are not totally able to avoid bubble formation, they may be able to avoid damaging massive bubbling. Decompression bubbles generally tend first to appear in lipid-rich tissue (*7, 9*), and if such tissue is in the acoustic pathway of a dolphin approaching a dangerous condition, the animal's high-frequency sounds could give warning to guide surfacing. Decompression bubbles, a very small fraction of a wavelength in diameter, are detectable in intact tissue by ultrasound (*7*). The partial opacity of the dolphin lower jaw to sound could, for example, prevent it from hearing clearly, because the jaw is in the sound pathway and is fat-filled. Guinea pigs have been successfully decompressed by keeping bubble size limited to about twice the diameter of capillaries (*9*); such bubbles were detected with 150-kHz sound, to which dolphins respond. In man symptomless bubbles occur, but the subjects are not aware of them in the way a dolphin might be.

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14 February 1980; revised 27 October 1980

Ridgway and Howard (*1*) showed that the partial pressure of dissolved N_2 in the muscles of dolphins that dive repeatedly to 100 m is about three times that in dolphins who remain at the surface. With this partial pressure in the tissues, a rapid ascent, or decompression, can produce the bends in man but not in dolphins or whales. That is, bubbles can form in blood, joint fluids, and at other sites, and they do so more easily in older persons.

In many respects, accounting for the difference between species is much like explaining what happens in a newly opened bottle of beer. If a bottle has been at rest for a day or so and is carefully opened, no bubbles appear in the resting beer. If the bottle has been shaken recently, or foreign matter is dropped into the fluid, bubbles evolve. The beer always bubbles when the flow is turbulent but not when the flow is smooth and laminar.

At a pressure of 4 or 5 atm, any bubbling in the charged beer must be due to heterogeneous nucleation, that is, the further filling of preexistent bubbles with the dissolved gases. Such pressure is not by itself sufficient to rend the attractive forces that hold water together. Accordingly, in the static case, we look to two sources for ebullition: free-floating "microbubbles" and vapor pockets at boundaries with solids. In respect of the latter, no solid surface in contact with beer is uniformly smooth microscopically. There are reentrant undercuts, patches that are nonwettable, sharp edges and points at grain boundaries, and all manner of favorable geometries to promotes separation of fluid from solid. At these places, particularly if there is a concavity of high curvature facing the beer, bubbles can form much more easily than in the fluid itself. [Surfactants such as foaming agents that reduce the surface tension of beer inhibit bubble growth in the presence of a preexisting trapped bubble (*2*). Surface-active chemicals, such as citrates, are used routinely to make long-lived club soda. But no surfactant can inhibit the growth of bubbles in solution.]

An experiment in our laboratory showed that shards of glass, grits, sand, and detritus of all sorts, when put into a dish of water before it is compressed to 4 or 5 atm, are not heterogeneous nucleators when the pressure is relieved after about 30 minutes. No bubbles appear

0036-8075/82/0507-0650$01.00/0