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Effect of milk on dopamine release in the newborn rat: an *in vivo* microdialysis study

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Newborn rats exhibit a rich behavioral repertoire to access the nipple and obtain milk. In older pups, catecholamines including dopamine (DA) mediate the behavioral effects of milk. In the present study, pups were delivered at term by caesarean section and instrumented with the microdialysis probe. Microdialysis samples were collected at 15 min intervals and K⁺-evoked levels of DA were measured with HPLC-ED. Pups received either no infusion, single or multiple intraoral infusions of saline or milk during subsequent samples. A decrease in K⁺-evoked DA release was evident after the first infusion in all subjects. Repeated milk infusions continued to reduce levels of extracellular DA, which remained evident 30 min after the last milk infusion. The rat neonate's first exposure to milk exerts lasting effects on neostriatal DA activity in the absence of prior suckling experience.

The behavior of the newborn mammal revolves around its need to obtain milk as the source of nutrient, mineral and water balance. Milk can serve as a potent sensory stimulus that promotes changes in motor activity, sensory responsiveness and learning in young animals⁷. The first presentation of milk to the perinatal rat reorganizes motor activity, evokes a stereotypic stretch response, and reduces responsiveness to thermal or tactile stimulation^{2,8}. The neurochemical changes that accompany these behavioral effects are less well documented.

After birth, intraoral infusion of milk produces an increase in motor activity that appears to be mediated by catecholamines. Behavioral activation is expressed by 6-day-old pups following milk infusion or administration of L-DOPA. Further the dopamine antagonist haloperidol blocks the effects of milk on general motor activity⁴. Other pharmacological manipulations of the dopamine system can promote behavioral changes in term rat fetuses that mimic the effects induced by milk⁸. The present study describes the effects of one or multiple infusions of milk on dopamine release in caesarean-derived newborn rat pups, which lack prior

experience with milk. *In vivo* microdialysis^{1,5} was used to sample extracellular dopamine from the neostriatum of rat pups following infusion.

Newborn rats were produced by Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) bred in groups of three at constant room temperature (22°C), and 12-h light:12-h dark cycle (lights on at 07.00 h). Pups were delivered by caesarean section at term (21 days after sperm were detected in a vaginal smear). Following delivery, pups were lightly tapped to facilitate respiration and group-housed in a moist incubator at 32.5°C². Rats were maintained following guidelines for animal care established by the National Institutes of Health (PHS publication 86-23).

One pup from each litter was selected for surgery, placed in an ice bath to effect anesthesia, and fitted with a microdialysis probe (Carnegie Medicin, CMA/12, Stockholm, Sweden). The probe, which exhibits a relative recovery rate of 10% at a flow rate of 5 μ l/min, was inserted at coordinates (from bregma): A 1.2 mm, L 1.2 mm, V 3.8 mm, with the head in a level position and cemented into place using dental acrylic and cyanoacrylate. The pup was placed in a 32.5°C

testing environment and permitted to recover from cold anesthesia. Within 10–15 min, prepared pups exhibited activity levels comparable to unoperated littermates.

The microdialysis probe was perfused continuously with artificial cerebrospinal fluid (aCSF concentration in mM: NaCl 143.5, CaCl₂ 3.0, KCl 4.0, MgCl₂ 1.0 and phosphate buffer 1.2; with a pH of 7.4) at a rate of 5 μ l/min. This aCSF has been found to facilitate developmental analysis of DA release⁶. After postsurgical recovery and collection of 3 baseline samples, the probe was perfused with normal aCSF for 0.5 min, an isotonic, high potassium (50 mM) aCSF for 4 min, then normal aCSF for the remainder of the 15-min sample interval (Fig. 1). High-K⁺ pulses were used to evoke DA release⁹, increasing accuracy of measuring DA in newborn pups. CSF solutions were changed during sampling with a liquid switch (Carnegie Medicin CMA/110). Samples were collected in microcentrifuge tubes containing 10 μ l of 0.6 M perchloric acid, 0.01% sodium metabisulfite and 10⁻⁶ M dihydroxybenzylamine.

DA was quantified by high performance liquid chromatography with electrochemical detection (HPLC-ED)⁶. Compounds were separated on a Biophase II column (Bioanalytical Systems; C₁₈, 3 μ m particle size), with a mobile phase consisting of a buffer containing 0.1 M NaH₂PO₄, 0.1 mM EDTA, 0.15% OSS and 6.5% MeOH (adjusted to a pH of 3.5). The flow rate was 1 ml/min. The detection system used a glassy carbon electrode maintained at a potential of +0.6 V vs. Ag/AgCl.

Neonatal subjects were assigned to 5 treatment groups ($n = 3$ per group). K⁺-evoked samples were collected during 6 15-min periods (K1–K6) from subjects in the no infusion group. Samples were collected from the remaining 4 groups following intraoral infusion of 30 μ l of isotonic saline or milk (bovine light cream) delivered in a 5-s pulse. Pups in the 2 groups presented with saline received a single infusion at the beginning of K3, or multiple infusions delivered at the beginning of K3–K5 (Fig. 1). The 2 groups exposed to milk similarly received either a single infusion at K3 or multiple infusions during K3–K5. Infusions were not delivered during the last sampling period (K6). At the conclusion of microdialysis sampling, the subject was euthanized by decapitation. In all subjects reported in this paper, the tip of the probe was verified by histological examination to be located in the neostriatum.

A repeated measures ANOVA compared levels of extracellular DA during 3 periods of basal release (B1–B3) and 2 periods of K⁺-evoked release (K1–K2). This analysis indicated the significant main effect of

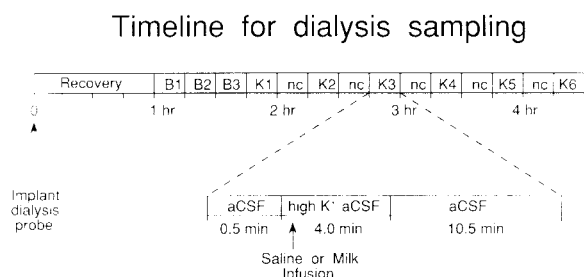


Fig. 1. Schematic outline of experimental design. After 1 h postsurgical recovery, 3 consecutive 15-min samples of basal release were collected (B1–B3). Immediately after the third basal sample, a series of 6 K⁺-evoked samples were collected (K1–K6). Each 15-min sample involved perfusion of normal aCSF for 0.5 min, high K⁺ aCSF for 4.0 min, and normal aCSF for the remaining 10.5 min of the interval. K⁺-evoked samples were separated by 15-min intervals in which no samples were collected (nc). At K3, subjects were presented with a single 30 μ l intraoral infusion of saline or milk at the beginning of the sample interval. Additional infusions were delivered to half the subjects at the beginning of K4 and K5. No infusions were delivered during the last sample interval (K6).

sample intervals, $F_{4,56} = 4.0$, $P < 0.01$. Post hoc comparisons of each interval indicated no change in DA release during the three basal samples (B1 = 0.65 ± 0.21 , B2 = 0.60 ± 0.10 , B3 = 0.57 ± 0.15 pmol/75 μ l), or between the 2 K⁺-evoked samples (K1 = 1.12 ± 0.28 , K2 = 1.03 ± 0.21 pmol/75 μ l; P values > 0.05). High-K⁺ aCSF significantly increased DA; K⁺-evoked samples were approximately double the level found in basal samples (P values < 0.05).

Measurements of extracellular DA collected during sample interval K2 were used to normalize measurements within individual subjects from all subsequent sample intervals (K3–K6). A one-way, repeated measures ANOVA indicated no significant change in extracellular DA levels during sample intervals K3–K6 in the no infusion group (K3 = $97.6 \pm 5.6\%$, K4 = $110.6 \pm 10.6\%$, K5 = $96.0 \pm 5.0\%$, K6 = $117.7 \pm 25.5\%$; $P > 0.50$). DA levels in the 4 chemosensory infusion groups, expressed as a percentage of interval K2, were analyzed in a 3-factor ANOVA, involving 2 substances (saline or milk) by 2 schedules (single or multiple infusions) by interval (K3–K5), with intervals treated as a repeated measure. This analysis indicated the significant main effect of substance, $F_{1,8} = 10.6$, $P < 0.05$ (Fig. 2), and no other effects. Milk resulted in significantly lower levels of DA than saline.

A planned comparison of extracellular DA levels during K3 was conducted with 3 groups: no infusion ($n = 3$), saline infusion ($n = 6$), and milk infusion ($n = 6$). This ANOVA indicated the significant main effect, $F_{2,12} = 6.7$, $P < 0.05$; subjects in the milk and saline groups exhibited reduced levels of DA than subjects which received no infusion. Saline and milk groups did not differ, implying that newborn rats respond to their

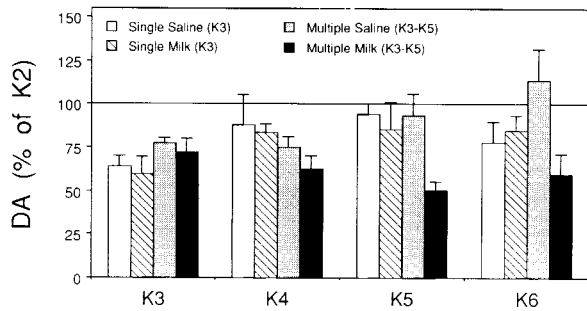


Fig. 2. Levels of extracellular DA among neonatal rats exposed to saline or milk infusion. Subjects were exposed to either one or a series of 3 infusions during intervals K3–K5, with no infusion delivered during K6. Bars represent the mean change in extracellular DA (\pm S.E.M.), expressed as a percentage of DA release during K2 (represented by the horizontal 100% line).

first intraoral infusion irrespective of the fluid's chemosensory properties.

Subjects received no infusion during interval K6. DA release during K6 was compared in a 2-factor ANOVA, which revealed the significant interaction of substance and schedule of infusion, $F_{1,8} = 5.5$, $P < 0.05$ (Fig. 2). Subjects exposed to a series of 3 milk infusions exhibited significantly lower levels of extracellular DA than subjects that received repeated infusions of saline. Milk infusions thus exert protracted effects on the neostriatal DA system.

Near-term fetal and neonatal rats exhibit behavioral and physiological responses to intraoral infusion of milk. Caesarean derived newborns, which lack prior postnatal experience with any fluid in the oral cavity, exhibit increased motor activity, aversive responses including facial wiping⁸, and bradycardia amounting to a 20–25% reduction from baseline HR after intraoral infusion of milk or saline. These behavioral and cardiac responses are rapidly modified with additional experience; pups show fewer aversive responses and less pronounced bradycardia to a second milk infusion or to their first exposure to milk if preceded by an initial infusion of saline⁸. These findings suggest that rat pups quickly adjust to repeated presentations of chemosensory stimuli on the day of birth.

The present study provides parallel evidence that the dopamine system is responsive to the neonate's first chemosensory experience and that responsiveness changes over a short series of infusions. Levels of extracellular DA are sharply reduced following the first infusion of either saline or milk. Among subjects that

receive only a single infusion, extracellular DA levels return to preinfusion levels within 30 min. Repeated experience with saline also results in extracellular DA levels that return to baseline by the last infusion. However, reduced DA release continues to be evident after repeated presentations of milk. These alterations in dopamine responsiveness are persistent, with multiple milk infusions resulting in lower extracellular DA levels 30 min after the last infusion.

Milk plays a fundamental role in regulating the behavior and physiology of mammals. Neonates of various species exhibit changes in activity and behavioral state that are related to cycles of suckling³. Even a single exposure to milk in the fetus, which lacks prior experience with this biologically important fluid, reduces behavioral responsiveness to other forms of stimulation and alters the temporal patterning of motor activity over a period of 30 min⁸. The finding that repeated milk infusion results in lasting changes in DA release implies that the dopamine system may be involved in mediating some of the protracted behavioral effects evoked by milk.

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