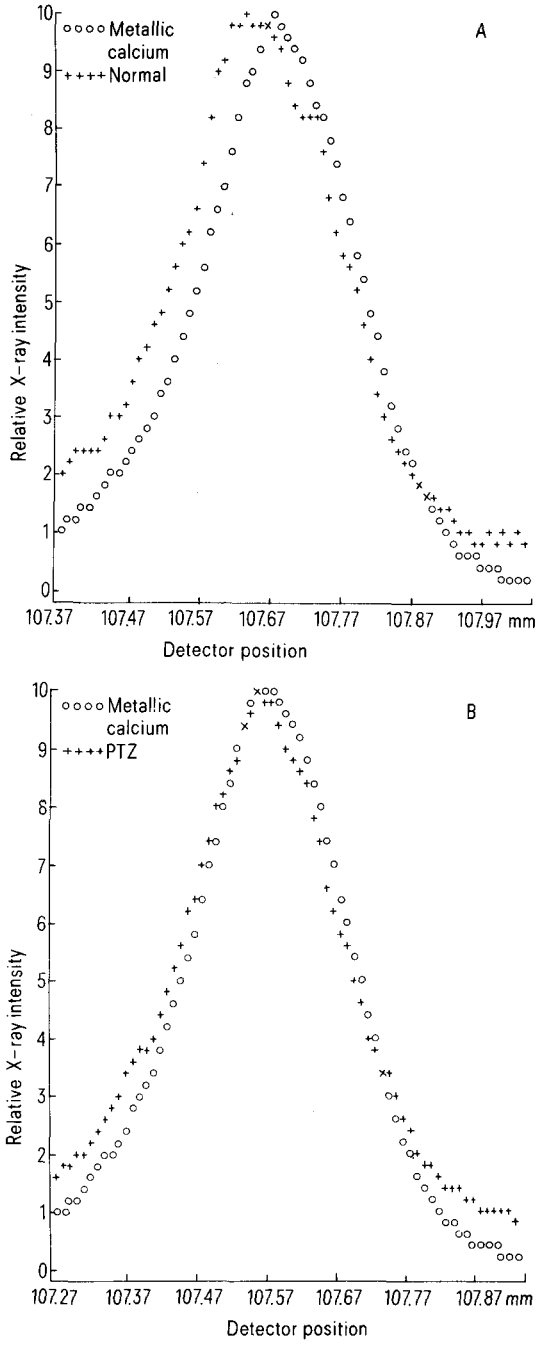

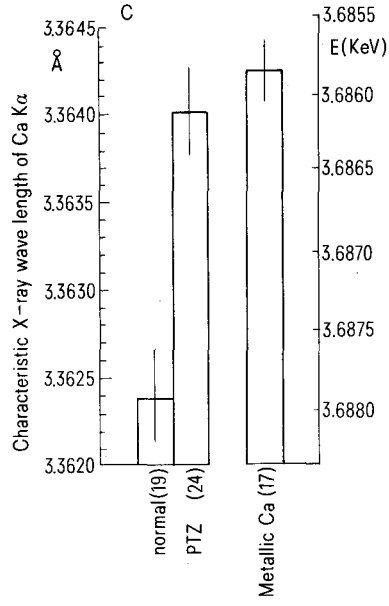

Chemical shift of calcium in normal and PTZ-treated freeze dried neurons. *A* An example of secondary X-ray intensity drawing by stepwise (10 μm) analysing crystal position movement (total of 67 steps) of metallic calcium (○) and normal neuron (+). The symbol × means the doubled position of the symbol ○ and +. Note the peak position shift of the normal neuron from the metallic calcium. *B* Same drawing but with metallic calcium (○) and PTZ-treated neuron (+). Note almost the same peak position between the two specimens. *C* Summary of chemical shift of the normal and PTZ-treated neurons. Values are mean ± SD. Numbers of experimental specimens are in parentheses.

like granules[6], and that membrane calcium density was remarkably increased during PTZ-induced bursting activity[7]. The above findings suggest that the increased calcium attached to the membrane area showed a different binding state. The significance of this change is completely obscure at this stage and requires further investigation.

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Intramembraneous particle change during pentylenetetrazole-induced bursting activity in snail neurons

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Summary. The freeze fracture study of snail neurons showed that during bursting activity induced by pentylenetetrazole, the intramembraneous particles demonstrated a different pattern from that of normal neurons.

When seizure discharge is produced in mammalian cerebral cortex by various methods such as pentylenetetrazole (PTZ) administration[1], penicillin application to the cerebral cortex[2] or repetitive cortical stimulation[3], the intracellular potential of the cerebral cortical neuron shows characteristic bursting activity (BA). Exactly the same pattern of BA can be seen when PTZ is applied to the specific neurons of the snail[4–6].

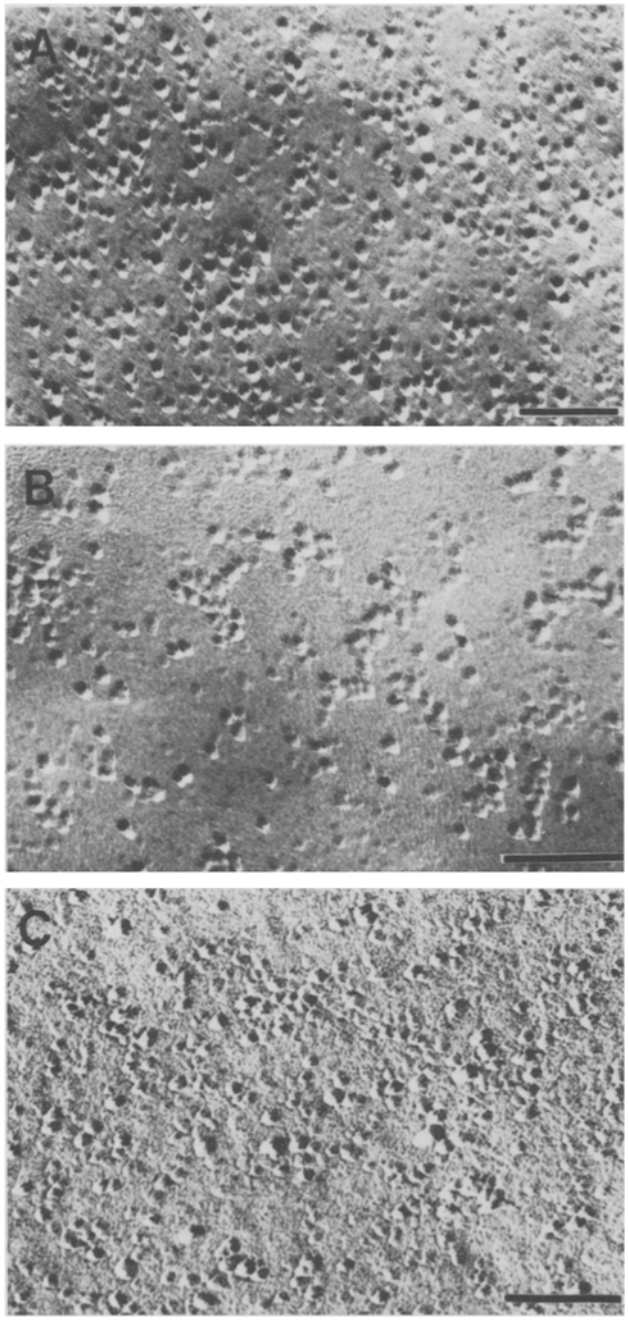

Figure 1. The freeze fracture replica of a snail neuron membrane. *A* Normal neuron. *B* After 15 min of incubation in 5×10^{-2} M PTZ. *C* After 30 min of rinsing with normal snail Ringer following 15 min of incubation in PTZ. Bars: 1 µm.

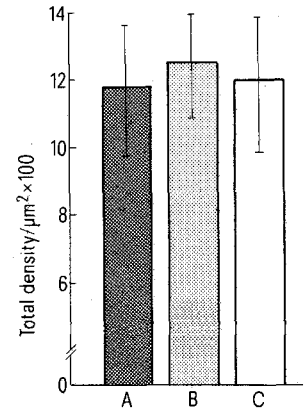

Figure 2. Densities of intramembraneous particles. *A* Normal state. *B* After PTZ incubation. *C* Recovery by rinsing with normal snail Ringer. Numbers ± SD (N = 6) in µm².

When BA was induced in D-neurons of the Japanese land snail, *Euhadra peliomphala,* the intracellular calcium shifted toward the cell membrane; this was shown by examination using a computer controlled electron probe X-ray microanalyzer (CCEPXMA)[7]. In the case of *Euhadra* D-neurons, the intracellular source of calcium is probably lysosome-like granules[8]. A combined study using CCEPX-MA and ion shower milling revealed that the shifted calcium is attached to the inner surface of the cell membrane[9]. If the shifted calcium is bound to some structure of the cell membrane such as intramembraneous protein, morphological changes of the cell membrane should be seen. The present study was undertaken to examine the intramembraneous morphological differences between normal neuronal membrane and that during PTZ-induced BA. The RC clusters of the suboesophageal ganglion of the Japanese land snail, *Euhadra peliomphala,* were used. The D- and H-neurons of the *Euhadra* RC cluster manifest prominent BA 5–10 min after application of 5×10^{-2} M PTZ[6]. The ganglia of the RC cluster were divided into 3 groups, 1 of which was incubated in 5×10^{-2} M PTZ in snail Ringer for 15 min; the 2nd group was incubated in normal snail Ringer, and the 3rd group was incubated in PTZ for 15 min and then rinsed with normal snail Ringer for 30 min. The specimens were fixed for 3 h by 5% glutaraldehyde in phosphate buffer (pH 7.2, 0.2 M) at 2 °C and then 40% glycerol (pH 7.2, 0.2 M) treatment was performed for 24 h. The ganglion was placed on a specimen holder and frozen in pool of freon 22 surrounded by liquid nitrogen. The specimen was placed in a JEE-5B vacuum evaporator (−190 °C, 10^{-6}–10^{-7} torr). Fracturing was carried out at about −120 °C with the FED-B fracture apparatus of JEOL. Double coating with carbon and platinized palladium was performed. A JEM-200CX electron microscope was used to study the replica at a 200 kV accelerating voltage.

Figure 1A shows the freeze fracture replica of normal neuron cell membrane. Intramembraneous particles 70–140 nm in diameter were scattered evenly. Figure 1B shows the freeze fracture replica of the PTZ-treated specimen. The intramembraneous particles of PTZ treated cell membrane showed an aggregated pattern. Figure 1C shows the cell membrane recovered from the PTZ effect by rinsing with normal snail Ringer for 30 min. To find some clue to show that the intramembraneous particles showed an aggregated pattern because of the movement of particles, the particle densities in a unit square were counted. The result is shown in figure 2 which indicates that the number of intramembraneous particles was almost the same in normal and PTZ-treated cells. This suggests that particles probably moved during BA.

The RC-cluster of *Euhadra* contains many D- and H-neurons which show BA by PTZ. Besides D- and H-neurons, there are a few I-neurons which never show BA by PTZ. In this experiment, we used the whole RC cluster because of technical problems. The possibility of fracturing the I-neuron was avoided by the positioning of the specimens in the fracture apparatus. Therefore, the above mentioned results should have been obtained on D- or H-neurons.

The previous work showed that the shifted intracellular calcium formed a new binding state[10]. In the examination by CCEPXMA and ion shower milling, the location of the shifted calcium was suggested to be at the inner surface of the cell membrane[9]. The above findings suggest that the intramembraneous particles treated with PTZ are in a different state from those in the normal neuronal membrane. The significance of this change is at present completely obscure and requires further study.

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Colon absorption of water and NaCl in the rat during lactation and the possible involvement of prolactin[1]

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Summary. The absorption of water and NaCl by the ascending colon of female rats was significantly increased by prolactin treatment in virgin rats and during suckled lactation. Bromocriptine treatment of lactating rats resulted in decreased colonic absorption, suggesting that increased prolactin secretion may be responsible for the enhanced colonic absorption seen during lactation.

The mammalian colon plays an important role in the maintenance of body fluid and electrolyte balance[4,5], by protecting the animal against excessive enteric losses of water and salts[6-9]. The possibility has been raised that variations in the endocrine status of the female mammal occurring during pregnancy and lactation[10-12] may be responsible for, or could contribute to, the observed hypertrophy and the elevated absorptive functions in the small intestine[11-13]. Since lactation is known to impose an additional burden on the water requirements of the mother, changes aimed at water and mineral ion conservation may be expected to occur in transporting epithelia such as the kidney tubule and gut during lactation[10]. However, information on the absorptive functions of the mammalian colon, during lactation seems to be lacking. Since prolactin has been shown to increase water and NaCl absorption in the ascending colon of rats[14], the elevated circulating levels of this hormone seen in lactation may contribute significantly to colonic absorption of water and ions in suckling animals.

The present study was therefore undertaken to investigate the influence of lactation and that of bromocriptine (CB-154), a potent prolactin secretion inhibiting agent[15,16], on the ability of the rat colon to absorb water and ions.

Materials and methods. The experiment was carried out using adult virgin mixed cycle and lactating Sprague Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) with body weights varying from 250 to 350 g. They were maintained on regular Purina rat chow and water was given ad libitum. Lactating rats were received 7 days before delivery, and at birth the litter size for each rat was adjusted to 9 or 10.

Each virgin rat was injected s.c. either with 1.0 mg ovine prolactin (oPRL:NIH-P-S10, 26 IU/mg) for 2 days or with hormone vehicle 48 and 24 h before absorption rates were studied.

Each lactating rat was also injected s.c. for 6 days beginning day 0 postpartum, with either 1.0 mg CB-154 per day or with the drug vehicle. Bromocriptine (CB-154) (Sandoz Ltd, Basel, Switzerland) was pepared for injection by dissolving the weighed amount in a small volume of 95% ethanol, into which an equal weight of tartaric acid was added, and warming gently. This was then diluted in a 1:20 ratio with 0.9% NaCl.

From nembutal-anesthetized animal 10-cm long everted colonic sacs were prepared from the ascending colon beginning 5 cm from the caecal-colonic junction.

Each sac was weighed empty and after it had been filled with aerated Krebs bicarbonate Ringer solution containing 10 mM D-glucose. Incubation of the sacs was done in 20 ml of the Ringer contained in a 50-ml Erlenmeyer flask, placed in a metabolic incubator and shaken at 80 oscillations/min. The contents of each flask were continuously bubbled with 95% O_2 : 5% CO_2 and maintained at 37 °C for 1 h.

Effect of ovine prolactin (oPRL), lactation and bromocriptine (CB-154) on the absorption of water, sodium and chloride by the rat ascending colon (mean ± SEM)

Treatment and number of animals	Mucosal fluid transport (ml/g wet wt/h)	Mucosal Na^+ transport (μEq/g wet wt/h)	Mucosal Cl^- transport (μEq/g wet wt/h)
Virgin rats			
Saline (5)	0.82 ± 0.04	146.6 ± 7.4	153.5 ± 6.3
1.0 mg oPRL (6)	1.14 ± 0.10[a]	194.4 ± 16.1[a]	204.9 ± 13.4[a]
Lactating rats			
Saline (11)	1.26 ± 0.04[b]	230.8 ± 8.9[b]	215.7 ± 6.5[b]
1.0 mg CB-154 (8)	0.80 ± 0.08[c]	150.1 ± 14.5[c]	145 ± 12.8[c]

[a] and [b] are significantly different from control virgin rat values ($p < 0.05$ and $p < 0.001$, respectively) [c] significantly different from control lactating rat values ($p < 0.001$).