# $^{45}Ca^{++}$ and $^{3}H$-GABA TRANSPORT IN NERVE ENDINGS ISOLATED FROM THE CEREBRAL CORTEX OF HYPOPARATHYROID RATS

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The clearest manifestation of hypofunction of the parathyroid glands, or hypoparathyroidism (HPT), is the syndrome of tetany, the neurochemical mechanisms of which have received special investigation only in recent times [1, 5-7, 9]. Our own neurophysiological studies showed [3] that hyperactivity of the central structures in HPT is due not only to hypocalcemia [10] and hypoxia [4, 6], but also to failure of inhibitory mechanisms [8]. This last effect may be linked with a disturbance of function of the nerve endings which secrete inhibitory amino acids.

To test this hypothesis, in the investigation described below uptake of $^{45}Ca^{++}$ and voltage dependent release of $^{3}H$-GABA by nerve endings isolated from the cerebral cortex of healthy rats and rats with HPT were studied.

## EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 160-180 g. HPT was induced by electrocoagulation of the parathyroid glands. The animals took part in the experiments 7-12 days after the operation, after all of them had developed a clinical picture of locomotor disorders and a lowered serum $Ca^{++}$ concentration, determined with the aid of a kit of reagents from Chemapol (Czechoslovakia).

Synaptosomes were isolated from the brain of healthy and hypoparathyroid rats simultaneously in one rotor of the centrifuge. Uptake of $^{45}Ca^{++}$ was investigated in synaptosomes, and $^{3}H$-GABA transport in the fraction of unpurified synaptosomes. Methods of studying the transport of these labeled compounds were fully described previously [2].

## EXPERIMENTAL RESULTS

Hypocalcemia is one of the chief consequences of a disturbance of parathyroid gland function. In this investigation injury to the parathyroid glands invariably led to a fall of the serum $Ca^{++}$ level: $1.45 \pm 0.04$ mM in HPT compared with $2.23 \pm 0.06$ mM in healthy animals ($p < 0.01$).

Investigation of $^{45}Ca^{++}$ uptake by the synaptosomes showed that the fastest rate of uptake during HPT was observed in the course of the first second; both at 37°C and at 0°C the rate of $^{45}Ca^{++}$ uptake was the same in the control as in the experiment. However, the subsequent course of $^{45}Ca^{++}$ accumulation by brain synaptosomes from rats with HPT differed appreciably from that in the control. This difference depended on temperature. The level of $^{45}Ca^{++}$ accumulation by synaptosomes of healthy rats, incubated in normal medium at 37°C, was near the steady state after only 1 min, for the increase in the $^{45}Ca^{++}$ concentration between 1 sec and 1 min differed only a little from the increase between 1 sec and 30 min (Table 1). The kinetics of $^{45}Ca^{++}$ uptake by synaptosomes at 0°C was different in character. The $^{45}Ca^{++}$ concentration in the synaptosomes 1 min after its addition showed only a very small increase compared with that observed after 1 sec, but it continued to rise, and after 30 min it was higher than at 37°C. In HPT, $^{45}Ca^{++}$ uptake by the synaptosomes was higher within the 1 sec—30 min interval than in the control, at both 37°C and 0°C. Not only the quantitative, but also the

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TABLE 1. $^{45}Ca^{++}$ Uptake (in nmoles/mg protein) by Cerebral Cortical Synaptosomes from Normal Rats and Rats with HPT (M ± m)

| KCl, mM | Time interval | 37 °C ($n$=5) | | 0 °C ($n$=4) | |
|---|---|---|---|---|---|
| | | control | HPT | control | HPT |
| 5 | 0—1 sec | 0,62±0,02 | 0,68±0,04 | 0,59±0,08 | 0,64±0,10 |
| | 1 sec—1 min | 0,43±0,04 | 0,51±0,03 | 0,10±0,07 | 0,12±0,06 |
| | 1 sec—30 min | 0,50±0,08 | 0,75±0,09* | 0,92±0,10 | 1,39±0,13* |
| 40 | 0—1 sec | 0,87±0,06 | 1,01±0,10 | 0,75±0,04 | 0,96±0,17 |
| | 1 sec—1 min | 0,47±0,08 | 0,58±0,14 | 0,59±0,18 | 0,47±0,17 |
| | 1 sec—30 min | 0,46±0,07 | 0,55±0,18 | 1,00±0,11 | 1,45±0,09* |

Legend. *$p < 0.05$ compared with control.

qualitative difference in the character of $^{45}Ca^{++}$ accumulation by the synaptosomes in HPT must be mentioned. Accumulation of $^{45}Ca^{++}$ by synaptosomes of rats with HPT at 37°C, like uptake of $^{45}Ca^{++}$ by control synaptosomes at 0°C, continued even after the first minute. In this case the increase in the $^{45}Ca^{++}$ concentration in the synaptosomes within the 1 sec—30 min interval was significantly greater than within the 1 sec—1 min interval (0.75 ± 0.09 and 0.51 ± 0.03 nmoles/mg protein respectively, $p < 0.05$).

At 37°C depolarization of the synaptosomes was accompanied by a significant increase in the fast (during the first second) $^{45}Ca^{++}$ uptake by synaptosomes in both the control and the experiment (Table 1, $p < 0.05$), and had a lesser effect on the rate of subsequent $^{45}Ca^{++}$ accumulation in the synaptosomes. This last observation is valid also for $^{45}Ca^{++}$ accumulation at 0°C.

The increase in $^{45}Ca^{++}$ uptake by the synaptosomes with an increase in the extracellular $K^+$ concentration is due not only to membrane depolarization, but also to other mechanisms [12]. Since $Ca^{++}$ entry into the cells through voltage-dependent Ca-channels is sensitive to a fall of temperature, an attempt was made to assess its contribution under normal conditions and in HPT, by comparing $^{45}Ca^{++}$ uptake at 37 and 0°C. It will be clear from Table 1 that the temperature-dependent component of $^{45}Ca^{++}$ entry into the synaptosomes in medium with a high $K^+$ concentration, i.e., the difference between $^{45}Ca^{++}$ uptake at 37 and 0°C, was greater in the control than in the experiment. For instance, in the four experiments in which data were obtained at both temperatures, the increase in $^{45}Ca^{++}$ uptake by control synaptosomes at 37°C (41.5 ± 8.0%) was significantly higher than at 0°C (20.2 ± 1.8%), whereas in the experiment the difference was not significant (46 ± 10 and 32 ± 10%, respectively).

In the next series of experiments uptake and release of $^3$H-GABA by unpurified synaptosomes were studied. No significant changes in the ability of the nerve endings to take up $^3$H-GABA could be discovered in HPT in medium both with and without $Ca^{++}$ (Table 2). So that the small differences in $^3$H-GABA uptake did not affect assessment of the influence of HPT on $^3$H-GABA release, the results of measurement of $^3$H-GABA secretion were given as percentages of the concentration of the transmitter in the synaptosomes. Release of $^3$H-GABA by synaptosomes from the brain of rats with HPT into medium of normal composition, containing $Ca^{++}$ ("leakage") was somewhat higher than the control in the case of preincubation in $Ca^{++}$-containing medium (Table 2), but the differences observed are not significant. On the other hand, comparison of the quantities of $^3$H-GABA released in response to depolarization (preincubation in $Ca^{++}$-containing medium) revealed a marked difference. Whereas depolarization led to a significant increase in transmitter release by synaptosomes of healthy animals ($p < 0.05$), release of $^3$H-GABA by synaptosomes of rats with HPT was significantly less than in the control, and remained essentially at the level of "leakage" of the transmitter in normal medium.

It will be clear from the account given above that HPT induces appreciable changes in the function of nerve endings, which persist even after isolation of the nerve endings from the brain, and can be detected under conditions when there is no difference in extracellular $Ca^{++}$ concentration. HPT evidently does not lead to very abrupt changes in the barrier properties of the plasma membrane of nerve endings: in short time intervals no increase in $^{45}Ca^{++}$ uptake by synaptosomes could be detected (Table 2). Nevertheless, the kinetics of subsequent accumulation of $^{45}Ca^{++}$ is evidence of an increase in the intrasynaptosomal reserves of $Ca^{++}$. In this respect the effect of HPT is opposite to the action of parathyroid hormone, which stimulates the rapid inflow of $^{45}Ca^{++}$ into the synaptosomes, whereas the steady-state level of $Ca^{++}$ accumulation differs from the control by a lesser degree [2].

TABLE 2. $^3$H–GABA Transport in Cerebral Cortical Synaptosomes of Normal Rats and Rats with HPT (M ± m)

| Parameter studied | Preincubation medium | | | |
|---|---|---|---|---|
| | without $Ca^{2+}$ ($n=4$) | | 0,1 mM; $Ca^{2+}$ ($n=5$) | |
| | control | HPT | control | HPT |
| Uptake, nmoles/mg protein | 0,65±0,07 | 0,71±0,15 | 0,51±0,02 | 0,44±0,02 |
| Release ("leakage"), % of uptake | 20,20—2,04 | 21,62—1,99 | 22,62—0,47 | 19,90—0,84 |
| Discharge on depolarization, % of uptake | 24,08±1,74 | 20,89±1,71 | 28,15±0,84* | 22,10±1,30 |

Legend. *$p < 0.01$ compared with "leakage."

Changes in $Ca^{++}$ uptake by synaptosomes in HPT may be connected with a change in the functional state of the mitochondria, which in HPT contain increased quantities of $Ca^{++}$ and synthesize smaller amounts of ATP [9]. The first factor must facilitate the more rapid accumulation of $^{45}Ca^{++}$ by a mechanism of homoexchange, whereas the second interferes with $Ca^{++}$ release from the synaptoplasm, which is a function of the ATP concentration in synaptosomes [10].

The most important result of the investigation of $^3$H-GABA transport in HPT is the discovery of inhibition of functionally important (voltage-dependent) release of the transmitter. This effect cannot be explained by a decrease in the inflow of $Ca^{++}$ into the synaptosomes, for under potassium depolarization conditions, an approximately equal quantity of $Ca^{++}$ enters the nerve endings in the control and in the experiment (Table 1). Perhaps due to an increase in the $Ca^{++}$ concentration in the synaptoplasm there is an increase in the release of transmitter at rest and a decrease in the reserves of easily released transmitter. This hypothesis is supported by the tendency for $^3$H-GABA uptake to be reduced in a medium containing $Ca^{++}$ (Table 2), by the increase in the frequency of the miniature end-plate potential compared with the control in the rat neuromuscular junction, isolated from an animal with HPT and incubated in medium with a normal $Ca^{++}$ concentration [2]. A decrease in sensitivity of the mechanism of transmitter secretion to intracellular $Ca^{++}$ under HPT conditions likewise cannot be ruled out. Whatever the case, reduction of the secretory activity of inhibitory nerve endings may be responsible for the phenomenon of absence of central inhibition and hyperactivity of the CNS in hypoparathyroidism.

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