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EFFECTS OF 24R,25-DIHYDROXYVITAMIN D_3 ON γ-GLUTAMYL-TRANSPEPTIDASE AND ALKALINE PHOSPHATASE ACTIVITIES IN RAT BRAINS

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Introduction. γ-Glutamyl transpeptidase (GGT) and alkaline phosphatase (AP) are two marker enzymes of the blood-brain barrier (BBB) which plays a major role in the control of brain homeostasis. Do *et al.* (1) have recently demonstrated that intraperitoneal (IP) injections of 24R,25-dihydroxyvitamin D_3 (24,25-$(OH)_2$ D_3) induce an increase in GGT and AP activities in rat kidneys. It was then of interest to investigate whether these enzymatic activities could also be modified in rat brains by vitamin D_3 metabolites. In addition to the effects of 24,25-$(OH)_2$ D_3, already demonstrated in the kidney, we are also studying those of 1,25-$(OH)_2$ D_3, considered as the biologically active metabolite. Since rat enzymatic activities are more elevated during night than during day (2), the experiments were carried out throughout the night period.

Materials and Methods. Female Sprague-Dawley rats (250 ± 30 g) were conditioned for 3 weeks before experiments at 20 ± 2 °C in artificial lighting from 8.00 a.m. to 8.00 p.m. and in darkness during the remaining period. Six animals per series received at various times during the night a single IP injection of 24,25-$(OH)_2$ D_3, 1,25-$(OH)_2$ D_3 or vehicle alone (control rats). Brains were removed from 6.00 a.m. to 8.00 a.m., when enzyme activities are maximum, then homogeneized in a potter device. After centrifugation at 2000 g for 20 min., the activities of GGT (GGT kit, Boehringer) and of AP (Enzyline kit, Biomérieux) were tested in the supernatants together with the protein concentration (Bio-Rad Laboratories).

Results and Discussion. In preliminary experiments, rat brains were collected 6 h after the IP injection of various doses of 24,25-$(OH)_2$ D_3 (from 5 to 100 ng/g.b.w.). Whereas the lower doses stimulated GGT and AP activities, the highest doses displayed inhibitory properties. Further experiments were carried out with the physiological dose of 10 ng/g.b.w.
Kinetic studies showed that 24,25-$(OH)_2$ D_3 induces in brain a constant increase in GGT activity from 2 h to 5 h, with a maximum of 67 % at 5 h (Fig. 1A), and a diphasic increase in AP activity (Fig. 1B). In contrast to that observed in the kidney (3), 24,25-$(OH)_2$ D_3 stimulates in the brain more intensely GGT than AP activity.
Interestingly, the IP injection of 10 ng/g.b.w. of 1,25-$(OH)_2$ D_3 induced first a sharp increase (up to 140 % at 3 h) followed by a decrease (-67 % and -77 % at 8 h and 24 h) in GGT cerebral activity. Although these data need to be confirmed by additional experiments, they suggest a down-regulation of GGT activity induced by 1,25-$(OH)_2$ D_3. In contrast, AP activity was constantly enhanced from 3 h to 6 h,

reaching its maximum level (50%) at 4 h, and then returned to its basal level.

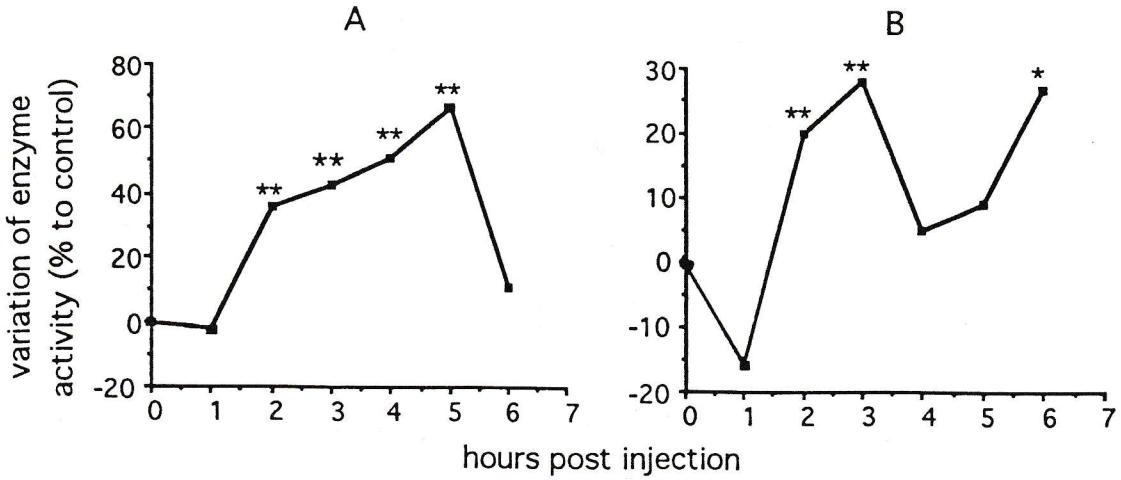

Figure 1: Kinetic effects of 24,25-$(OH)_2$ D_3 on GGT (A) and AP (B) activities in rat brains (Student's t-test: * $p < 0.05$, ** $p < 0.01$).

These results clearly demonstrate that both 24,25-$(OH)_2$ D_3 and 1,25-$(OH)_2$ D_3 are active on cerebral GGT and AP activities. This is of particular interest in the case of GGT, likely involved in the aminoacid transport through the BBB (4). In addition, the action of 1,25-$(OH)_2$ D_3 on the central nervous system (CNS) has been recently evidenced by in vitro (5) and in vivo (6) studies. Considering that both GGT and 1,25-$(OH)_2$ D_3 could be involved in the physiopathology of the CNS, their interrelationships should be further investigated.

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