Biochem. J. (1978) **170**, 445-448
Printed in Great Britain

Calcitonin-Mediated Changes in Plasma Tryptophan and Brain 5-Hydroxytryptamine and Acetylcholinesterase Activity in Rats

By ATIF M. NAKHLA and ADHIP P. NANDI MAJUMDAR*
Institute of Medical Biochemistry, University of Aarhus, DK-8000 Aarhus C, Denmark

(Received 16 *November* 1977)

After a single injection of calcitonin (20 M.R.C. units/kg body wt.) marked decreases in both Ca^{2+} and free tryptophan in plasma were observed, during the initial period of the treatment (up to 1 h). However, 5-hydrotryptamine contents of the whole brain and the cerebral acetylcholinesterase activity were greatly enhanced. The cerebellar acetylcholinesterase activity was not influenced by calcitonin.

The rate of 5-hydroxytryptamine (serotonin) synthesis in the brain is known to be dependent on the availability of the precursor amino acid tryptophan. However, the uptake of tryptophan into the brain is governed by the concentrations of several other neutral amino acids, and the concentration of tryptophan that is not bound to the blood protein fractions (for review see Wurtman & Fernstrom, 1976). Haymovits *et al.* (1976) have demonstrated that in hibernating bats a high thyroidal calcitonin concentration is associated with an increased 5-hydroxytryptamine content in the gland. During this hibernation period the animals also suffer from hypocalcaemia. Their observation suggests that the peptide hormone calcitonin may play a role in the mobilization of 5-hydroxytryptamine in tissues.

The intraperitoneal administration of tryptophan increases the concentrations of both tryptophan and 5-hydroxytryptamine in the brain (Fernstrom & Wurtman, 1971*a*). We have demonstrated that in both intact and adrenalectomized rats force-feeding of tryptophan markedly enhances cerebral acetylcholinesterase activity (acetylcholine hydrolase, EC 3.1.1.7) (Majumdar & Nakhla, 1977). In adrenalectomized rats tryptophan also enhances acetylcholinesterase activity in the heart, liver, lungs and spleen (Nakhla & Majumdar, 1977). Since an increase in plasma tryptophan is associated with high 5-hydroxytryptamine content in the brain (Fernstrom & Wurtman, 1971*a*; Munro *et al.*, 1975), our observation of higher acetylcholinesterase activity in the brain after tryptophan force-feeding could in part be attributed to an increase in 5-hydroxytryptamine in the brain. These findings suggest that changes in the concentration of either tryptophan or 5-hydroxytryptamine in the brain affect the activity of acetylcholinesterase in this tissue. This, and the possibility that calcitonin might regulate the 5-hydroxytryptamine concentration in certain tissues, prompted us to investigate (*a*) whether administration of calcitonin would cause any change in plasma tryptophan and brain 5-hydroxytryptamine contents, and (*b*) whether any such change would affect acetylcholinesterase activity in either cerebral or cerebellar regions of the brain.

* To whom reprint requests should be addressed.

Materials and Methods

Male Wistar rats weighing between 150 and 200 g were maintained on food and water *ad libitum*. The animals were injected intramuscularly with either pig calcitonin (10.25 M.R.C. units/mg; Armour Pharmaceutical Co., Eastbourne, Sussex, U.K.) in gelatin diluent or an equivalent volume of gelatin diluent only. The rats were killed at different intervals. Blood was collected in heparinized centrifuge tubes and plasma was obtained by centrifugation at 5000 rev./min for 15 min in a Sorvall centrifuge. The brains were quickly removed, and cerebral hemisphere and cerebellum were dissected out and frozen immediately on solid CO_2.

Total plasma calcium was determined with a Unicam SP.90 atomic absorption spectrophotometer according to the manufacturer's instruction manual. Tryptophan concentration in the protein-free plasma fraction was determined by the norharman fluorescence method of Denkla & Dewey (1967) as modified by Lehmann (1971) and Bloxam & Warren (1974). The concentration of 5-hydroxytryptamine in the brain was measured by the procedure described by Curzon & Green (1970).

Acetylcholinesterase activity in the cerebral and cerebellar regions of the brain was determined by the method of Ellman *et al.* (1961) with acetylthiocholine as substrate, as reported previously (Majumdar & Nakhla, 1977).

For statistical analysis non-paired Student's *t* test was used.

Results

Effect of a single injection of calcitonin on total plasma calcium, over a period of 12h

The responsiveness of the rat to pig calcitonin was studied by measuring the total plasma Ca^{2+} concentration after a single injection of calcitonin (20 M.R.C. units/kg). This dose was chosen from a dose–response study, where it was found to produce a maximum decrease (30–35%) in plasma Ca^{2+} after 1h treatment (results not shown). The results shown in Fig. 1 revealed that the total Ca^{2+} in plasma decreased rapidly after a dose of calcitonin. At 1h after the injection, plasma Ca^{2+} concentration was decreased by about 30%, remained at that value for another 1h, and then began to increase again, returning to its normal value 12h after calcitonin injection.

Effect of calcitonin on free tryptophan in plasma and on whole-brain 5-hydroxytryptamine concentrations

A single injection of calcitonin produced an immediate and pronounced decrease in free tryptophan in plasma, whereas 5-hydroxytryptamine content in the brain during this period was markedly increased (Fig. 2). At 1h after calcitonin injection the concentration of free tryptophan in plasma was 35% lower than in the control. But the values returned to the control value within the next hour, and after another 4h the tryptophan concentration was 26% above the control value and then started to decline slowly. The brain 5-hydroxytryptamine content, on the other hand, was increased by 56% 30min after calcitonin injection, and then returned essentially to its control value over the next 1.5h and increased again, until at 12h after the hormone treatment it was 64% higher than in the control (Fig. 2).

Dose and time-course responsiveness of cerebral and cerebellar acetylcholinesterases to calcitonin

The responsiveness of acetylcholinesterase to increasing doses of calcitonin was first investigated. All animals were killed 1h after the hormone injection. Whereas none of the doses (1, 5, 10, 20, 50 or 100 M.R.C. units/kg) used in the present study caused any alteration in the activity of cerebellar acetylcholinesterase, a 26% enhancement of the cerebral enzyme was observed after a dose of

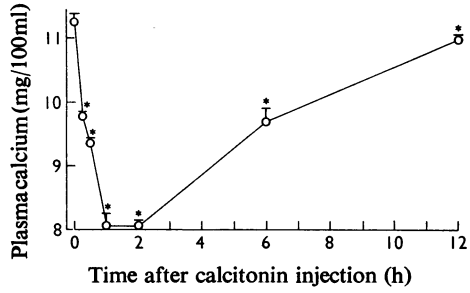

Fig. 1. *Effect of calcitonin on total plasma calcium concentration*
Groups of three or four rats were killed at different times after an intramuscular injection of pig calcitonin (20 M.R.C. units/kg). Blood from each rat was collected individually and plasma calcium concentration was determined. Each value on the curve represents the mean±S.E.M. $*P<0.001$, as compared with the zero-time control.

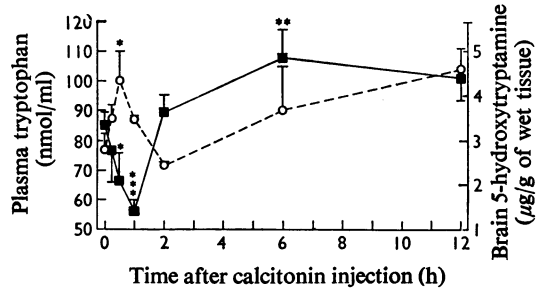

Fig. 2. *Time-course changes in the concentration of free tryptophan* (■) *in plasma and the brain 5-hydroxytryptamine* (○) *content after calcitonin injection*
Experimental procedure was the same as described in Fig. 1. Each value represents the mean±S.E.M. $*P<0.05$, $**P<0.025$, $***P<0.001$, as compared with the zero-time control.

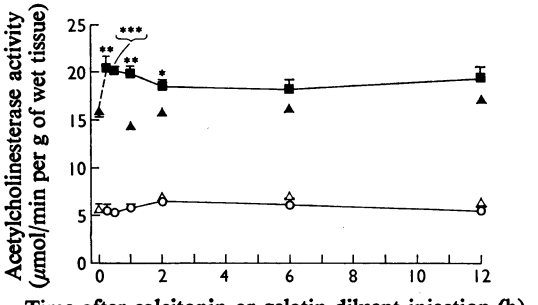

Fig. 3. *Effect of calcitonin on the activity of acetylcholinesterase in the cerebral hemisphere* (■) *and cerebellum* (○) *of the brain, and the enzyme activity in the cerebral hemisphere* (▲) *and cerebellum* (△) *after gelatin-diluent injections*
Groups of four rats were injected with either calcitonin (20 M.R.C. units/kg) or gelatin diluent only, and were killed at different intervals. Each value represents the mean±S.E.M. $*P<0.025$, $**P<0.005$, $***P<0.001$, as compared with the zero-time control.

20 M.R.C. units/kg (results not shown): this dose was also most effective in lowering the plasma Ca^{2+} concentration.

The time course of the activity of both cerebral and cerebellar acetylcholinesterase was then investigated after calcitonin injection. The results shown in Fig. 3 revealed that a single injection of calcitonin (20 M.R.C. units/kg) produced an immediate and pronounced enhancement in the activity of cerebral acetylcholinesterase. At 15 min after calcitonin treatment cerebral acetylcholinesterase showed 28% higher activity than in the control. The activity then declined slowly over the next 1.45 h; at 2 h after calcitonin treatment the cerebral acetylcholinesterase showed 16% higher activity than control, and remained essentially at that value for the rest of the experimental period. On the other hand, no significant change in the activity of cerebellar acetylcholinesterase was observed over the 12 h experimental period (Fig. 3).

To determine whether diurnal variation might influence cerebral or cerebellar acetylcholinesterase activities, control rats were killed at different times after administration of gelatin diluent only. No significant variation in the activity of the enzyme in either cerebral or cerebellar regions was observed (Fig. 3).

Discussion

The results of the present investigation show that a single injection of calcitonin to normal rats produces an immediate decrease in total calcium and free tryptophan in plasma, with a concomitant rise in both brain 5-hydroxytryptamine content and the cerebral acetylcholinesterase activity.

The concentrations of several neutral amino acids and of free tryptophan (i.e. not bound to plasma proteins) are known to influence the uptake of tryptophan into the brain (Blasberg & Lajtha, 1965; Fernstrom & Wurtman, 1971*a*,*b*). In the present investigation we have measured only the concentration of free tryptophan in plasma, which would be utilized by the brain for 5-hydroxytryptamine synthesis. The concentration of 5-hydroxytryptamine in the brain was measured to correlate the changes in plasma free tryptophan with 5-hydroxytryptamine after calcitonin injection. Our observation that during the initial period of calcitonin treatment (up to 1 h) there is a sharp decrease in free tryptophan in plasma, with a concomitant rise in 5-hydroxytryptamine content in the brain, suggests that calcitonin enhances 5-hydroxytryptamine accumulation in the brain, and, in part, could be attributable to an increased availability of free tryptophan in plasma. These changes occurred at a time when the plasma calcium content was also greatly decreased (Fig. 1). Whether a decrease in plasma calcium by other means would also produce similar changes in the brain 5-hydroxytryptamine and plasma tryptophan contents remains to be elucidated.

It has been shown that administration of DL-5-hydroxytryptophan increases 5-hydroxytryptamine in the brain, and this increase is temporally correlated with certain behavioural changes (Aprison & Hingtgen, 1966, 1970; Aprison *et al.*, 1975). In our attempt to study the responsiveness of the brain to tryptophan under different physiological conditions, we have measured acetylcholinesterase activity in the brain. The results of our earlier experiments (Majumdar & Nakhla, 1977) show that administration of tryptophan, a condition that is shown to increase 5-hydroxytryptamine in the brain (Fernstrom & Wurtman, 1971*a*; Munro *et al.*, 1975), stimulates acetylcholinesterase activity in the brain. That tryptophan stimulates cerebral but not cerebellar acetylcholinesterase activity (Majumdar & Nakhla, 1977) suggests further than the enzyme of the different regions does not respond similarly to tryptophan stimulus. In the present investigation a similar phenomenon was observed. A single injection of calcitonin that produced an increase in the content of brain 5-hydroxytryptamine also stimulated acetylcholinesterase activity in the cerebral hemisphere, but not in the cerebellum (Fig. 3). It appears therefore that the enhancement of cerebral acetylcholinesterase activity after calcitonin injection is the result of the calcitonin-mediated increase in 5-hydroxytryptamine in the brain.

Lastly, it should be mentioned that a single injection of pentagastrin, the bioactive *C*-terminal tetrapeptide of gastrin, has been found to stimulate cerebral acetylcholinesterase activity (A. P. N. Majumdar & A. M. Nakhla, unpublished work). This, together with the above observation, indicates that the brain acetylcholinesterase is responsive to a number of polypeptide hormones.

A. M. N. is the recipient of a post-doctoral fellowship from DANIDA, Ministry of Foreign Affairs, Denmark. The project was partly supported by grants to A. P. N. M. from the Statens Laegevidenskabelige Forskningsråd (J.nr. 512-6545 and 512-8111) and DANIDA, Denmark.

References

Aprison, M. H. & Hingtgen, J. N. (1966) *Life Sci.* **51**, 1971–1978

Aprison, M. H. & Hingtgen, J. N. (1970) *Int. Rev. Neurobiol.* **13**, 325–341

Aprison, M. H., Hingtgen, J. N. & McBride, W. J. (1975) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 1813–1822

Blasberg, R. & Lajtha, A. (1965) *Arch. Biochem. Biophys.* **112**, 361–377

Bloxam, D. L. & Warren, W. H. (1974) *Anal. Biochem.* **60**, 621–625

Curzon, G. & Green, R. A. (1970) *Br. J. Pharmacol.* **39**, 653–655
Denkla, W. D. & Dewey, H. K. (1967) *J. Lab. Clin. Med.* **69**, 160–169
Ellman, G. L., Courtney, D., Anders, V., Jr. & Featherstone, R. M. (1961) *Biochem. Pharmacol.* **7**, 88–95
Fernstrom, J. D. & Wurtman, R. J. (1971*a*) *Science* **173**, 149–152
Fernstrom, J. D. & Wurtman, R. J. (1971*b*) *Science* **174**, 1023–1025
Haymovits, A., Gershon, M. D. & Nunez, E. A. (1976) *Proc. Soc. Exp. Biol. Med.* **153**, 383–387
Lehmann, J. (1971) *Scand. J. Clin. Lab. Invest.* **28**, 49–55
Majumdar, A. P. N. & Nakhla, A. M. (1977) *Biochem. Biophys. Res. Commun.* **76**, 71–77
Munro, W. N., Fernstrom, J. D. & Wurtman, R. J. (1975) *Lancet* **i**, 722–724
Nakhla, A. M. & Majumdar, A. P. N. (1977) *Biochem. Biophys. Res. Commun.* **79**, 96–104
Wurtman, R. J. & Fernstrom, J. D. (1976) *Biochem. Pharmacol.* **25**, 1691–1699