agités pendant 8 h. Après centrifugation (2000 × g) et filtration, on recueille la solution éthanolique. Cette opération est répétée 2 fois (1,5 l et 1 l). Les solutions hydro-alcooliques sont concentrées à demi-volume et extraites par le benzène. La phase aqueuse est acidifiée par HCl jusqu'à concentration 4 N puis chauffée au bain-marie (100 °C, 4 h).

Les génines, qui précipitent, sont recueillies par filtration (5,85 g). Ce précipité est repris par le benzène bouillant; la partie soluble (4,75 g) est chromatographiée sur Alumine Merck (activité II-III, 140 g). L'élution (fractions de 50 ml) par benzène, benzène-éther et éther fournit 4 produits qui cristallisent du méthanol. Des fractions benzène on recueille le produit **1** (1,91 g, F 232-233 °C, sublimé), des fractions benzène-éther (90:10) les composés 2 (30 mg, F 295 °C, sublimé) et **3** (15 mg, F 231-232 °C) et des fractions éther le produit **4** (80 mg, F 200 °C).

Tableau 2. Spectre de RM^{1}H de **4**

	H-11	H-12	H-19	H-21	H-26 et H-27	H-28	H-29	H-30
$CDCl_3$	5,12	4,38	1,19	1,60	0,87	1,00	0,91	0,82
C_6D_6	5,42	4,85	1,43	1,83	0,87	1,18	1,04	0,92
C_5D_5N	5,60	4,80	1,38	1,94	0,87	1,24	1,08	1,00

Les spectres sont enregistrés à 60 MHz sur spectromètre Varian T60. Les déplacements chimiques sont exprimés en ppm, le Me_4Si servant d'étalon interne.

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Pentagastrin induction of acetylcholinesterase activity in cerebral cortex of rats

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Summary. A single injection of pentagastrin (500 μg kg^{-1}) produced an immediate (within 15 min) and pronounced increment (about 50%) in the activity of acetylcholinesterase (AChE) in the cerebral but not in the cerebellar region of the brain. Pretreatment of rats with either actinomycin-D or cycloheximide did not fully abolish the pentagastrin-mediated stimulation of cerebral AChE activity.

The early observation that extracts of equine brain and intestine contain a peptide (now called substance P) with smooth muscle stimulating and hypotensive properties[2,3] has led to the search for other peptides common to the brain and gut. Somatostatin and vasoactive intestinal peptide (VIP) have also been shown to be present in brain and gut tissues[4,5]. Recently, using a number of antisera raised against octapeptide cholecystokinin (CCK 8) and gastrin, Dockray[6] has been able to demonstrate the presence of a CCK-like peptide in the cerebral cortex of rat, dog and hog. In addition, peptides similar to the antral hormone gastrin are also found to be present in certain parts of the brain[7]. Although the significance of the occurrence of gastrin and CCK-like peptides in the brain is not fully understood, a potential role as neutrotransmitters or neuromodulators has been suggested[6,8]. In an effort to study the responsiveness of the brain to gastrin and CCK-like hormones, the acetylcholinesterase (acetylcholine hydrolase, EC 3.1.1.7) activity in the cerebral hemisphere and cerebellum was investigated following a single injection of pentagastrin, a synthetic peptide containing the bioactive carboxy-terminal tetrapeptide portion of both CCK and gastrin.

Materials and methods. Adult male Wistar rats (150-200 g), fasted for 48 h, were injected i.p. with either saline or pentagastrin (peptavlon, I.C.I., Macclesfield, England) and killed at different intervals as stated in the text below. The cerebral hemisphere and cerebellum were dissected out, and were frozen immediately on solid CO_2. Acetylcholinesterase activity (AChE) in the cerebral hemisphere and cerebellum was measured by the method of Ellman et al.[9] as described earlier[10].

Results and discussion. In the 1st series of experiments, the responsiveness of AChE to increasing doses of pentagastrin was investigated after 1 h of the hormone treatment. Whereas none of the doses (50, 250, 500, 750 and 1000 μg kg^{-1}) used in the present study caused any alteration in the activity of cerebellar AChE, a 26% enhancement in the cerebral hemisphere was observed after a dose of 500 μg kg^{-1} (results not shown). Higher doses of pentagastrin were less effective in this respect.

The time interrelationship of the activity of AChE in both cerebral hemisphere and cerebellum was then investigated. A single injection of pentagastrin (500 μg kg^{-1}) produced an immediate and pronounced increment in the activity of the enzyme in the cerebral hemisphere (figure). A maximal stimulation of 50% over the control occurred 30 min after pentagastrin injection. The enzyme activity then declined rapidly, and at 2 h after pentagastrin treatment the values were essentially the same as that of the control, and remained at that level for the rest of the experimental

period (figure). Cerebral and cerebellar AChE activities in the control rats, measured at various intervals after saline injection, were not different from the corresponding initial values (figure), indicating that diurnal variation and prolongation of fasting period had no influence on the activity in either cerebral or cerebellar regions of the brain. There was also no change in AChE activity in the cerebellum after pentagastrin injection, over a period of 6 h (figure).

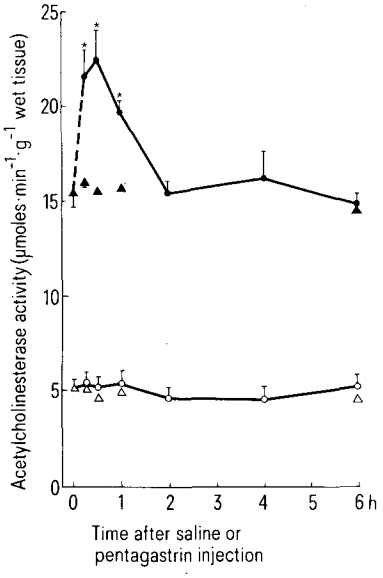
The stimulation of AChE activity in the cerebral hemisphere after pentagastrin injection could be the result of an increased synthesis of the enzyme or an activation of the existing enzyme molecules. In an effort to distinguish between these 2 possibilities, rats were injected with either cycloheximide or actinomycin-D 1 h before administration of pentagastrin. AChE activity was measured 30 min after the hormone treatment. 1.5 h after cycloheximide treatment [^{3}H]-leucine incorporation in vivo into total brain, protein was 80–85% decreased (data not shown), showing that the present dose of cycloheximide effectively blocks protein synthesis in the brain. The inhibition of RNA synthesis in the brain by actinomycin-D was not measured, but the dose chosen was the same as used earlier by us and other workers for similar experiments[10,11]. In the present experiment, as observed before (figure), administration of pentagastrin to saline-treated rats produced a 55% increment in the cerebral AChE activity, when compared with the control (table). However, when pentagastrin was injected to cycloheximide and actinomycin-D treated rats, the degree of stimulation was found to be reduced, but the hormone still caused 19 and 38% enhancements, respectively, compared to the non-antibiotic treated control (table). The observation that even in the absence of normal protein and RNA synthesis pentagastrin stimulates the cerebral AChE activity, though to a lesser extent, suggests that the hormone-mediated stimulation of the enzyme activity could be the result of both increased synthesis and activation of the enzyme.

In conclusion, the present experimental results demonstrate that a single injection of pentagastrin promptly stimulates AChE activity in the brain. The observation that pentagastrin enhances the cerebral but not the cerebellar AChE activity indicates that the enzyme of different regions of the brain does not respond similarly to pentagastrin stimulus. Interestingly, the cerebral cortex contains abundancy of CCK and gastrin-like peptides, whereas the cerebellum is practically devoid of these materials[7,12]. Furthermore, recent immunocytochemical studies have demonstrated the presence of CCK-like peptides in nerve terminals of the cerebral cortex (L.-I. Larsson and J.F. Rehfeld, personal communication). These, and the present observation of increased cerebral AChE activity after pentagastrin injection, suggest that one of the functions of gastrin and CCK-like peptides in the cerebral cortex is to regulate AChE activity. Whether changes in the endogenous level of CCK and gastrin would also alter AChE activity in various regions of the brain remains to be elucidated.

Effect of pentagastrin on cerebral acetylcholinesterase activity in cycloheximide and actinomycin-D treated rats

Treatment	Acetylcholinesterase activity (μmoles min^{-1} g^{-1} wet tissue)
Control (saline)	13.4±0.38 (100)
Pentagastrin	20.8±1.84 (155; $p<0.005$)
Cycloheximide + Saline	13.9±0.54 (104; NS)
Cycloheximide + Pentagastrin	15.9±0.40 (119; $p<0.001$)
Actinomycin-D + Saline	14.1±0.56 (105; NS)
Actinomycin-D + Pentagastrin	18.5±0.76 (138; $p<0.001$)

Actinomycin-D (1 mg kg^{-1}, Calbiochem, Lucerne, Switzerland) or cycloheximide (10 mg kg^{-1}, Sigma Chemical Co, St. Louis, USA) injected i.p. 1 h before administration of pentagastrin (500 μg kg^{-1}). The rats were killed 30 min after saline or pentagastrin injection. Each value represents the mean±SEM (n=10). The figures in the parentheses represent percentage of the saline-control as well as p-value. NS = not significant.



Effect of pentagastrin 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 saline injections. Rats were injected with either saline or pentagastrin (500 μg kg^{-1}), and were killed at different intervals. Each value on the curve represents the mean±SEM (n=6). *Significantly different compared to the respective saline control at the level of $p<0.001$ as judged by Student's t-test.

1 Acknowledgments. A.M. Nakhla is recipient of a postdoctoral research fellowship from Danida, Ministry of Foreign Affairs, Denmark. The project was in part supported by grants to A.P.N.M. from The Laegevidenskabelige Forskningsråd and Danida, Denmark.

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