The involvement of hormones in the regulatory mechanism of enzymatic development of intestinal brush border membrane has been established especially in the neonate[20]. Recently sucrase[21] maltase[22] and enterokinase[23] activities were induced precociously in rat embryos by administration to the mother of cortisone and/or thyroxine. In cultured chick embryo intestine, thyroxine is able to enhance the early accumulation of AlPase in the explants[24]. The present data show that EGF treatment increases AlPase and trehalase activities in fetal mouse small intestine in utero.

We have previously shown that endoplasmic reticulum membrane-bound glucose-6-phosphatase (G-6-Pase) activity develops late during the gestational period[25]. The daily administration of EGF during 3 days beginning at 15 days of gestation induces a significant increase of G-6-Pase activity in the proximal intestinal thirds of the fetuses at 18 days of gestation (controls: $(3.43 \pm 0.24)10^{-3}$; EGF-treated: $(5.10 \pm 0.40)10^{-3}$; 10 assays each; $p < 0.0025$). This observation is in agreement with the ability of EGF to induce in vitro the differentiation of the rough endoplasmic reticulum of fetal mouse absorptive cells[9]. This effect of EGF on intestinal G-6-Pase activity seems to be particular to the fetal period since it has no effect on this activity during the postnatal period when the endoplasmic reticulum is well developed[8].

In conclusion the present observations suggest that EGF may play a role in the overall maturation of absorptive cells in mouse fetus, as it does in the differentiation of the lung in the fetal rabbit[26]. EGF accelerates the differentiation of the endoplasmic reticulum in absorptive cells and induces in increase in some specific brush border enzyme activities.

1 Supported by grant MA-6069 from the Medical Research Council of Canada. Dr D. Ménard is a 'chercheur boursier du Fonds de la recherche en santé du Québec'.
2 S. Cohen, J. biol. Chem. *237*, 1555 (1962).
3 S. Cohen and G. Carpenter, Proc. natl Acad. Sc. USA *72*, 1317 (1975).
4 J.B. Elder, G. Williams, E. Lacey and H. Gregory, Nature *271*, 466 (1978).
5 L.A. Scheving, Y.C. Yeh, T.H. Tsai and L.E. Scheving, Endocrinology *105*, 1475 (1980).
6 L.A. Scheving, Y.C. Yeh, T.H. Tsai and L.E. Scheving, Endocrinology *106*, 1498 (1980).
7 E.J. Feldman, D. Aures and M.I. Grossman, Proc. Soc. exp. Biol. Med. *159*, 400 (1978).
8 C. Malo and D. Ménard, Gastroenterology, in press (1982).
9 J.-F. Beaulieu and R. Calvert, J. Histochem. Cytochem. *29*, 765 (1981).
10 O.H. Lowry, N.F. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
11 K. Burton, Biochem. J. *62*, 315 (1956).
12 K.W. Giles and A. Myers, Nature *4979*, 93 (1965).
13 S. Lloyd and W. Whelan, Analyt. Biochem. *30*, 467 (1969).
14 A. Dahlqvist, Analyt. Biochem. *7*, 18 (1964).
15 A. Eichholz, Biochim. biophys. Acta *135*, 475 (1967).
16 D. Ménard and C. Malo, Devl Biol. *65*, 508 (1978).
17 R. Calvert, D. Malka and D. Ménard, Cell Tissue Res. *214*, 97 (1981).
18 D. Ménard, C. Malo and R. Calvert, Devl Biol. *85*, 150 (1981).
19 C. Malo and D. Ménard, Experientia *35*, 874 (1979).
20 F. Moog, in Textbook of gastroenterology and nutrition in infancy, vol. 1, p. 139. Ed. E. Lebenthal. Raven Press, New York 1981.
21 P. Celano, J. Jumawan, C. Horowitz, H. Lau and O. Koldovsky, Biochem. J. *162*, 469 (1977).
22 J. Jumawan, P. Celano, C. Horowitz, H. Lau and O. Koldovsky, Biol. Neonate *32*, 211 (1977).
23 E. Lebenthal, Pediat. Res. *11*, 282 (1977).
24 B.L. Black and F. Moog, Devl Biol. *66*, 232 (1978).
25 R. Calvert, D. Malka and D. Ménard, Histochemistry *63*, 209 (1979).
26 W.Z. Catterton, M.B. Escobedo, W.R. Sexson, M.E. Gray, H.W. Sundell and M.T. Stahlman, Pediat. Res. *13*, 104 (1979).

Two cell types in monoamine-containing 'liquor contacting' neuron system of the frog brain

M. Yoshida, I. Nagatsu[1], Y. Kondo, N. Karasawa, T. Ohno, M. Spatz and T. Nagatsu

Department of Anatomy, School of Medicine, Fujita-Gakuen University, Aichi 470-11 (Japan), Laboratory of Neuropathology and Neuroanatomical Sciences, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda (Maryland 20205, USA), and Laboratory of Cell Physiology, Department of Life Chemistry, Graduate School at Nagatsuta, Tokyo Institute of Technology, Yokohama 227 (Japan), 26 February 1982

Summary. By means of an immunofluorescent technique, 'liquor contacting' neurons, serotonergic in nature were demonstrated in the paraventricular organ and in the nucleus infundibularis dorsalis, and neurons catecholaminergic in nature were noted in the preoptic recess organ and in the caudal part of the 4th ventricle.

The distribution of monoaminergic neurons in the amphibian brain has been demonstrated by means of formaldehyde-induced fluorescence methods[2-9]; catecholaminergic and serotonergic neuron systems were localized within specific areas of the CNS. In the amphibian brain, very strong monoamine fluorescence was found in the cells of 'circumventricular organs' such as the preoptic recess organ (PRO), paraventricular organ (PVO) and the lateral infundibular region or nucleus infundibularis dorsalis (NID). These cells were termed 'liquor contacting neurons' because of their intense contact with the cerebrospinal fluid[2]. These circumventricular organs have been known to contain green fluorescent neurons and a few yellow or greenish-yellow ones[6-8]. With the technique of microspectrofluorimetry, the fluorescence in PVO or NID was thought to be due to catecholamine (CA), probably dopamine, and serotonin (5-HT), and that of PRO to CA probably dopamine only[8].

We have now performed an immunofluorescent study in frogs using antibodies to CA-synthesizing enzymes, tyrosine hydroxylase (TH), and to 5-HT, in order to corraborate the findings obtained by the histofluorescence method and to provide an 'immunohistochemical map' of monoaminergic neuron systems with special reference to the liquor contacting neuron system.

Materials and methods. Antiserum to TH was produced in rabbits and tested for specificity as described previously[10]. Rabbit antiserum to 5-HT was purchased from RIA (UK). Frogs (*Rana catesbiana*; b.wt 300–450 g) were anesthetized with ether and perfused via the arterial trunk with Zamboni's solution (2% paraformaldehyde-0.2% picric acid in 0.1 M phosphate buffer, pH 7.2). The brains were removed and postfixed with the same fixative for 6-17 h. Subse-

quently they were washed, rinsed in phosphate buffer containing 10% sucrose, and frozen on dry ice. Frozen sections 10 µm in thickness were made in a cryostat and processed for the indirect immunofluorescent technique[10]. After immunofluorescent staining, the sections were mounted in buffered glycerol (0.5 M carbonate, pH 8.6-glycerol, 1:1) and examined with a Leitz Orthoplan fluorescence microscope, equipped with a Ploem vertical illuminator – an activation filter KP490, a red suppression filter BG38 and barrier filter K530 – with a mercury vapor lamp as the light source[11].

Results. In the medulla oblongata, TH-positive neurons were observed in the dorso-lateral part, namely in the nucleus tractus solitarius and in the neighbouring reticular formation. Apart from these neurons, some cells with TH-positive immunofluorescence found beneath and among the ependymal layer in the midline of the caudal part of the 4th ventricle (fig. 1,a and b). Some processes of these cells penetrated into the ventricular lumen, thus they appeared to belong to the liquor contacting neuron system. In turn, 5-HT-positive neurons were situated in the ventro-medial portion near the 4th ventricle but their processes made no direct contact with the cerebrospinal fluid (CSF) space (fig. 1,c). These neurons were found to appear at the level of the caudal part of the 4th ventricle and to continue rostrally as far as the isthmic tegmentum. At the level of the interpeduncular nucleus in the transverse section, 5-HT-positive neurons were scattered within the raphe region and the reticular formation, but no TH-positive neurons were seen in these areas. Small numbers of TH-positive neurons were found at the same level dorsally in the region ventral to the nucleus isthmii. In the rostral part of the midbrain tegmentum, TH-positive small neurons were observed in the basomedial portion in the nucleus reticularis mesencephalii.

In the diencephalon, TH- or 5-HT-positive neurons were noticed mainly in the periventricular area of the preoptic and infundibular regions. Small numbers of TH-positive

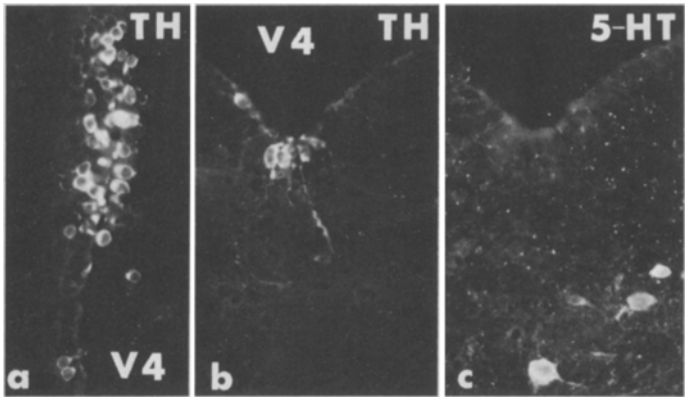

Figure 1. Immunofluorescent micrographs of 'liquor contacting' neurons situated in the caudal part of the 4th ventricle (V4). *a* Horizontal plane through the floor of the 4th ventricle showing numerous 'liquor contacting' neurons with TH-positive immunofluorescence (upwards is rostral, × 190); *b* and *c* consecutive transverse sections through the caudal brainstem; *b* TH-positive neurons were seen at the ventro-medial wall of the 4th ventricle (V4), and processes of these cells penetrate into the ventricular lumen (× 190); *c* 5-HT-positive neurons located in more ventral part, but no 5-HT-positive liquor contacting neurons was found at this level (× 190)).

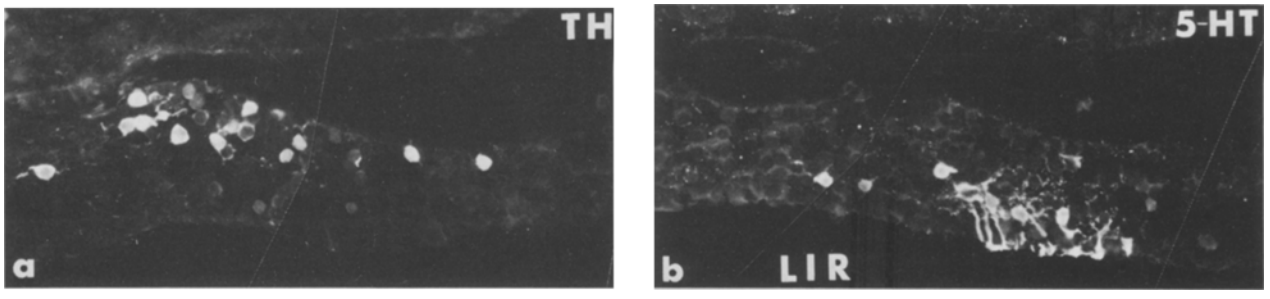

Figure 2. Immunofluorescent micrographs through the dorsal infundibular region in consecutive transverse sections. *a* TH-positive neurons are situated in the dorsal part of the NID mainly and these cells do not protrude with their processes into the ventricle (× 190); *b* 5-HT-positive liquor contacting neurons located in the dorsal margin of the lateral infundibular recess (LIR) in the NID (× 190).

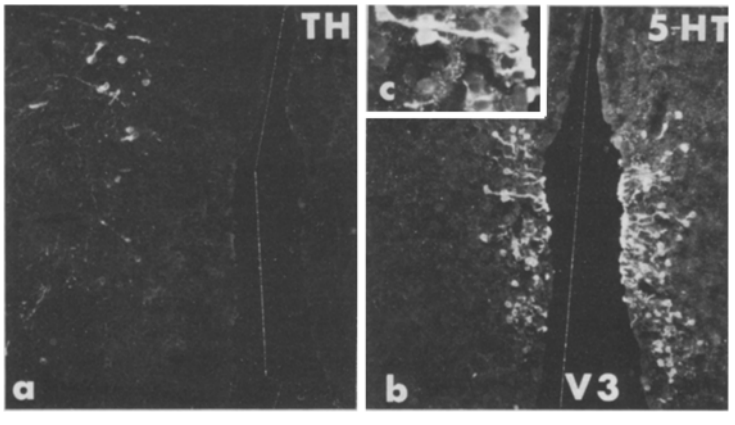

Figure 3. Immunofluorescent micrographs through the rostral part of the hypothalamus in the transverse plane. *a* No specific TH-positive fluorescence was seen in the PVO, but cells near the PVO (so-called PVO-accompanying cells) show TH-positive immunofluorescence (× 75); *b* 5-HT-positive immunofluorescent liquor contacting neurons in PVO (× 75); *c* high power magnification of 5-HT-positive cells in the PVO. Apical 'palisading' protrusions with specific fluorescence (× 190).

neurons were found in the pretectal area. In the transverse plane at the level of caudal hypothalamus, TH-positive neurons were localized in the dorsal part of the NID; yet, they were not found to protrude with their processes into the 3rd ventricle (fig. 2, a). After incubation of the consecutive section with 5-HT antiserum, 'liquor contacting' neurons with specific immunofluorescence appeared at the dorsal margin of the lateral infundibular recess (LIR) in the NID (fig. 2, b). In the more rostral sections of the hypothalamus, TH-positive neurons were found to be scattered in the area lateral to the PVO, the so called PVO-accompanying cells[6], but the liquor contacting neurons in the PVO lacked TH-positive immunofluorescence (fig. 3, a). The liquor contacting neurons of PVO were found to show 5-HT-positive immunofluorescence, but the PVO-accompanying cells were negative (fig. 3, b). 5-HT-positive processes of these cells penetrated into the 3rd ventricle and their intraventricular protrusions had strong immunofluorescence and formed palisades (fig. 3, c). In the preoptic region, TH-positive liquor contacting neurons were widely distributed between the lining of ependymal cells and in the subependymal layer (fig. 4, a). These cells were more abundant in the rostro-dorsal part than in the caudoventral part. Apart from these cells, TH-positive neurons were seen in the suprachiasmatic region and in the periventricular gray around the preoptic recess. However, 5-HT-immunofluorescent cells were not detected in these areas (fig. 4, b).

Discussion. The distribution of monoaminergic neuron systems, as elucidated in the present immunohistochemical study, generally agrees with previous findings obtained by histofluorescent techniques[2-9]. However, some differences and new findings were seen in the present study.

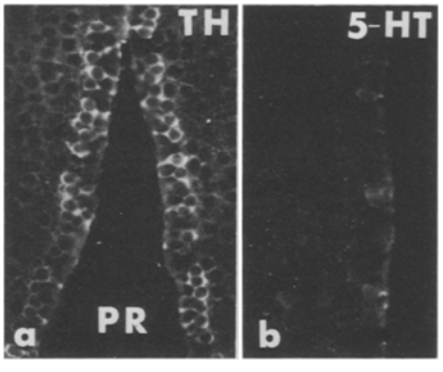

Figure 4. Immunofluorescent micrographs through the PRO in the transverse plane. *a* Numerous liquor contacting neurons with TH-positive immunofluorescence around the preoptic recess (PR) (× 190); *b* no 5-HT-positive immunofluorescence are present in the PRO (× 190).

1. In the caudal part of the 4th ventricle, there are TH-positive, CA-producing liquor contacting neurons but not 5-HT containing ones. 2. The liquor contacting neurons in the PVO and NID were found to be TH-negative but 5-HT-positive. 3. Small numbers of TH-positive neurons, but no 5-HT-positive neurons, were found in the pretectal area.

The liquor contacting neurons localized in the caudal brainstem, are similar in their morphology to the CA-producing, liquor contacting neurons in the PRO. Their localization suggests a phylogenetic relationship to the area postrema of mammals.

We also found besides 5-HT-containing liquor contacting neurons another type of neurons in the NID and around the PVO; especially in the NID, TH-positive neuronal perikaryas were noted to be situated near the 5-HT-positive liquor contacting neurons. Therefore, this region may be supplied with a specific innervation by both CA (dopamine) and 5-HT neurons.

From histofluorescent studies, it has been reported that 'liquor contacting' neurons of PVO contained both catecholamine and serotonin[2-9]. From our present immunofluorescent findings, it is now clear that 'liquor contacting' neurons of PVO contain only 5-HT but no TH-positive catecholaminergic neurons. This fact may suggest the possibility that monoamines (dopamine and 5-HT) were taken up from CSF into the liquor contacting neurons in PVO.

The functions of these 'liquor contacting' neurons are unknown. There may be 2 functions: 1. to discharge their neurosecretory material by way of an apocrine secretion from liquor contacting neurons into the ventricle; and 2. to receive information from the CSF that may influence the neurosecretory activity. Further studies are necessary to elucidate the physiological function of 'liquor contacting' neurons.

1 To whom reprint requests should be addressed.
2 I. Vigh-Teichmann, P. Röhlich and B. Vigh, Z. Zellforsch. *98*, 217 (1969).
3 B. Vigh and I. Vigh-Teichmann, Int. Rev. Cytol. *35*, 189 (1973).
4 H. Braak, Z. Zellforsch. *106*, 269 (1970).
5 A. Parent, J. Morph. *139*, 67 (1973).
6 M. Terlou and R.E. Ploemacher, Z. Zellforsch. *137*, 521 (1973).
7 T. Chacko, M. Terlou and J. Peute, Cell Tissue Res. *149*, 481 (1974).
8 P.D. Prasada, Rao and H.G. Hartwig, Cell Tissue Res. *151*, 1 (1974).
9 M. Tohyama, K. Yamamoto, K. Sato, T. Sakumoto and N. Shimizu, J. Hirnforsch. *18*, 223 (1977).
10 I. Nagatsu, Y. Kondo, S. Inagaki, N. Karasawa, T. Kato and T. Nagatsu, Acta histochem. cytochem. *10*, 494 (1977).
11 I. Nagatsu, Acta histochem. cytochem. *7*, 147 (1974).

A specific endogenous inhibitor of two forms of Ca^{++} activated neutral proteases in platelets

M. Sakon, T. Tsujinaka, J. Kambayashi and G. Kōsaki

Hemostasis Research Unit, The 2nd Department of Surgery, Osaka University Medical School, Fukushima-ku, Osaka 553 (Japan), 5 November 1981

Summary. An endogenous, specific inhibitor of high molecular weight has been isolated from bovine blood platelets, which inhibits the activity of the 2 forms of platelet Ca^{++} activated neutral proteases reported previously by us. The inhibition is not due to chelation of Ca^{++} but results from a stoichiometric complex formation.

The presence of a Ca^{++} activated neutral protease (CANP) has been reported in various tissues and it has been termed as KAF[1], CaAF[2], CANP[3], RTF[4]. Their physiological roles may consist in myofibrillar protein degradation[2,5] or activation of kinases[1,6], but these reported CANPs require a relatively higher concentration of Ca^{++}