ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 2000, 60: 209-214

Effects of pituitary adenylate cyclase-activating polypeptide (PACAP) on cyclic AMP formation in the duck and goose brain

Jerzy Z. Nowak, Katarzyna Kuba and **Jolanta B. Zawilska**

Department of Biogenic Amines, Polish Academy of Sciences, 3 Tylna St., P-225, 90-950 Łódź, Poland, Email: jerzyn@amina1.zabpan.lodz.pl

Abstract. Two molecular forms of pituitary adenylate cyclase-activating polypeptide (PACAP), i.e., $PACAP_{27}$ and $PACAP_{38}$ (0.0001-1 μM), as well as vasoactive intestinal polypeptide (VIP; 0.1-3 μM), have been studied for their effects on cyclic AMP formation in the hypothalamus and cerebral cortex of duck and goose. All three peptides concentration-dependently stimulated cyclic AMP production in the tested brain regions of 2-3-weeks-old (young) ducks, with VIP showing at least one order of magnitude weaker activity than PACAP. This characteristics suggests the existence in the duck's brain of adenylyl cyclase-linked PAC_1 receptors. Both forms of PACAP also stimulated the nucleotide formation in the cerebral cortex and hypothalamus of 5-6-months-old (adult) ducks or geese grown under natural environment. The peptides-evoked effects in adult and young ducks were comparable, and clearly greater than those found in adult geese. The present data extend our recent observations made on chicks, and suggest PACAP to be a potent stimulator of the cyclic AMP generation in the avian central nervous system.

Short communication

Key words: PACAP (pituitary adenylate cyclase-activating polypeptide), $PACAP_{27}$, $PACAP_{38}$, VIP (vasoactive intestinal polypeptide), cyclic AMP, hypothalamus, cerebral cortex, duck, goose

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a member of vasoactive intestinal polypeptide (VIP)/secretin/glucagon family (Christophe 1993, Arimura and Shioda 1995, Rawlings and Hezareh 1996). It exists in two biologically active molecular forms, PACAP 1-27 ($PACAP_{27}$) and PACAP 1-38 ($PACAP_{38}$), of which the latter is a predominating form occurring in tissues (Miyata et al. 1990, Arimura and Shioda 1995, Rawlings and Hezareh 1996). PACAP evokes many effects in a variety of tissues, and diverse roles the peptide may play - particularly in mammalian species - include the role of a central neurotransmitter or neuromodulator, endocrine regulator, vasoregulator, and also growth factor (Chartrel et al. 1991, Christophe 1993, Arimura and Shioda 1995, McRory et al. 1995, Harmar et al. 1998, Peeters et al. 1998a,b, Wong et al. 1998).

One of the most prominent biochemical actions of PACAP is its ability to stimulate the cyclic AMP-generating system. This effect results from the nature of PACAP receptors (designated PAC_1, $VPAC_1$ and $VPAC_2$ (Harmar et al. 1998)), which all belong to the large superfamily of G-protein coupled receptors linked to the activation of adenylyl cyclase (noteworthy, at least one of these receptors, i.e., PAC_1, can also couple to the activation of phospholipase C) (Christophe 1993, Arimura and Shioda 1995, Rawlings and Hezareh 1996, Harmar et al. 1998). PACAP-evoked elevations in cyclic AMP synthesis/content have been found in many tissues of different mammals (Miyata et al. 1990, Christophe 1993, Onali and Olianas 1994, Arimura and Shioda 1995, Basile et al. 1995, Nilsson et al. 1996, Rawlings and Hezareh 1996), as well as in brains of frogs (Yon et al. 1992, Chartrel et al. 1993) and fishes (McRory et al. 1995, Wong et al. 1998). Recently we have observed a stimulatory effect of the peptide on cyclic AMP formation in the central nervous system (CNS) of chicks (Nowak et al. 1999a,b), thus extending an array of vertebrates being sensitive to PACAP. In these birds, grown from the day of hatching under controlled lighting and temperature conditions, the action of $PACAP_{27}$ and $PACAP_{38}$ varied among tested brain areas, displaying large effects in the hypothalamus and cerebral cortex, and comparatively moderate effects in the pineal gland, and weak ones in optic lobes. In a recent communication Peeters et al. (1998b) also reported stimulatory effects of PACAP on cyclic AMP content in and growth hormone release from cultured chicken anterior pituitary cells. Our recent preliminary data have shown the ability of a short form of PACAP, i.e. $PACAP_{27}$, to enhance cyclic AMP synthesis in the brain of some other birds, such as duck, goose and turkey (Nowak et al. 1999b), suggesting that the peptide may generally play a neuromodulator role in the avian CNS. In this report we present our extended data on the effects of the two molecular forms of PACAP, i.e., $PACAP_{27}$ and $PACAP_{38}$, as well as VIP, on cyclic AMP formation in the hypothalamus and cerebral cortex of young and adult ducks, and, in addition, of adult geese.

Female 2-3-weeks-old (young) and 5-6-months-old (adult) ducks (*Anas platyrhynchos f. domestica*) and adult geese (*Anser anser f. domestica*) were used. Young ducks refer to the animals that were purchased locally on the day of hatching and kept until experimentation in warmed brooders under 12 h light: 12 h dark lighting schedule (LD; lights on at 22.00 h and off at 10.00 h), with a*d libitum* standard "avian" food and tap water. The adult animals, that were grown under natural environmental lighting and temperature conditions, were purchased (in November 1998) from local farmers; these animals were kept until sacrifice in an unheated room (average temperature of $11 \pm 3^{o}C$) under artificial illumination of 9 h light: 15 h dark (similar to a natural day-night cyclicity in November), with a free access to standard "avian" food and tap water. On the day of experiment, the birds (adult - three on each occasion; young - usually 4-5 animals per experiment) were killed by decapitation, under light, between 09.00-11.00. Each experiment was carried out on the hypothalamal tissue pooled from two-three animals and cerebral cortex (devoid of white matter) of one animal, and repeated 2-3 times. Cross-chopped slices (250 μm; prepared with McIlwain tissue chopper) of the tested brain regions were suspended in cold, O_2/CO_2 (95:5) gassed, glucose--containing modified Krebs-Henseleit medium (containing 118 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, and 11.7 mM D-glucose; pH 7.4). The formation of [^{3}H]cyclic AMP in [^{3}H]adenine prelabeled tissues was assayed according to Shimizu et al. (1969). The formed [^{3}H]cyclic AMP was isolated by a sequential Dowex-alumina chromatography according to Salomon et al. (1974). The results were individually corrected for a percentage recovery with the aid of [^{14}C]cyclic AMP added to each column system prior to the nucleotide extraction. The accumulation of cyclic AMP during a 10-min stimulation period was assessed as a per cent of the conversion of [^{3}H]adenine to [^{3}H]cyclic AMP. The effects of PACAPs and VIP were tested in the medium without any protease

inhibitors. Details of the whole procedure were described by us earlier (Nowak and Sek 1994, Nowak et al. 1995).

The following drugs were used: $PACAP_{27}$ (human, ovine, rat - P-202; RBI, Natick, MA, USA), $PACAP_{38}$ (mammalian - A-1439; Sigma, St. Louis, MO, USA) and VIP (human, porcine, rat -V-6130; Sigma, St. Louis, MO, USA). [^{3}H]Adenine (specific activity 26.9 Ci/mmol) and [^{14}C]cyclic AMP (specific activity 52.3 mCi/mmol) were purchased from DuPont New England Nuclear (Bad Homburg, Germany). Other chemicals were of analytical purity and were obtained mainly from Sigma (St. Louis, MO, USA).

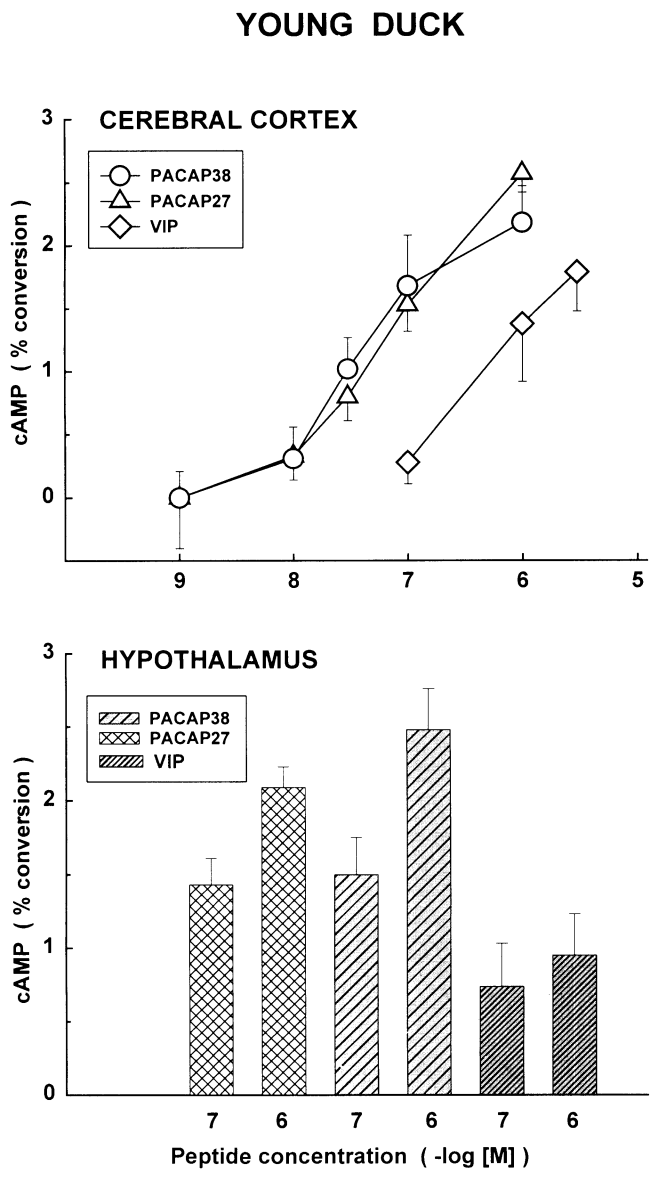

Fig. 1. The effects of $PACAP_{27}$, $PACAP_{38}$ and VIP on cyclic AMP formation in [^{3}H]adenine-prelabeled slices of the cerebral cortex and hypothalamus of young duck. Results are expressed in percent of conversion and represent means ± SEM, showing net effects of the peptides, i.e., after subtraction of respective basal (control) values which were (in percent of conversion): cerebral cortex, 2.03 ± 0.39(11); hypothalamus, 0.85 ± 0.17(9). Number of samples: 5-13 and 9-13 for cerebral cortex and hypothalamus, respectively.

Data were expressed as means ± SEM, and were analyzed by one-way analysis of variance followed by Newman-Keuls test, using GraphPad software.

Young ducks. $PACAP_{27}$ and $PACAP_{38}$, used in a concentration range of 0.001-1 μM, similarly and concentration-dependently stimulated cyclic AMP formation in cerebral cortex, producing at a 1 μM dose the effect of 207-227% of control. $PACAP_{27}$ and $PACAP_{38}$, applied at 0.1 and 1 μM concentrations, acted more strongly in the hypothalamus, producing increases in cyclic AMP production of 268-276 and 346-392% of control, respectively (Fig. 1). In parallel experiments, VIP (0.1-1 μM in the hypothalamus and 0.1-3 μM in cerebral cortex) appeared to be at least one order of magnitude weaker (than PACAP) stimulator of the nucleotide synthesis in the studied brain regions (Fig. 1). None of the tested peptides did not reach a plateau (indicating a maximal response) at the used concentration range, making impossible calculation of EC_{50} values (representing the concentrations required for half-maximal cyclic AMP production).

Adult ducks and geese. The obtained results are shown in Figs. 2 and 3. $PACAP_{27}$ (0.001-1 μM) and $PACAP_{38}$ (0.0001-1 μM) stimulated cyclic AMP production in a concentration-dependent manner in the hypothalamus and cerebral cortex of both adult duck and goose. Maximal responses (E_{max}) have been reached at 0.1 μM of both $PACAP_{27}$ and $PACAP_{38}$ in the goose hypothalamus (267 and 320% of control, respectively), with calculated EC_{50} values of 10.4 nM for $PACAP_{27}$ and 1.7 nM for $PACAP_{38}$, as well as in the duck cerebral cortex at 0.1 μM of $PACAP_{38}$ ($E_{max} \approx 300\%$ of control; $EC_{50} \approx 26.7$ nM). Both peptides at concentrations used did not reach a plateau in the duck hypothalamus. A raphly similar potency of $PACAP_{27}$ and $PACAP_{38}$ in evoking the cyclic AMP response in the duck hypothalamus and goose cerebral cortex was observed, whereas in the goose hypothalamus $PACAP_{38}$ appeared to be clearly more potent than its 27-form.

Interestingly, as demonstrated in our recent experiments carried out on chicks (Nowak et al. 1999a) both - short and long - forms of PACAP evoked decisively

larger cyclic AMP responses in the hypothalamus and cerebral cortex of these birds (E_{max} of 900-1,000 and 500-600% of respective control, reached in both cases at 0.1 μM concentration; with the exception of $PACAP_{38}$ in the hypothalamus where the peptide action did not approach a plateau until 1 μM), yet displaying comparable EC_{50} values (i.e., 26.9 and 26.3 nM, respectively, for $PACAP_{27}$, and 18.6 nM for $PACAP_{38}$ in cerebral cor-

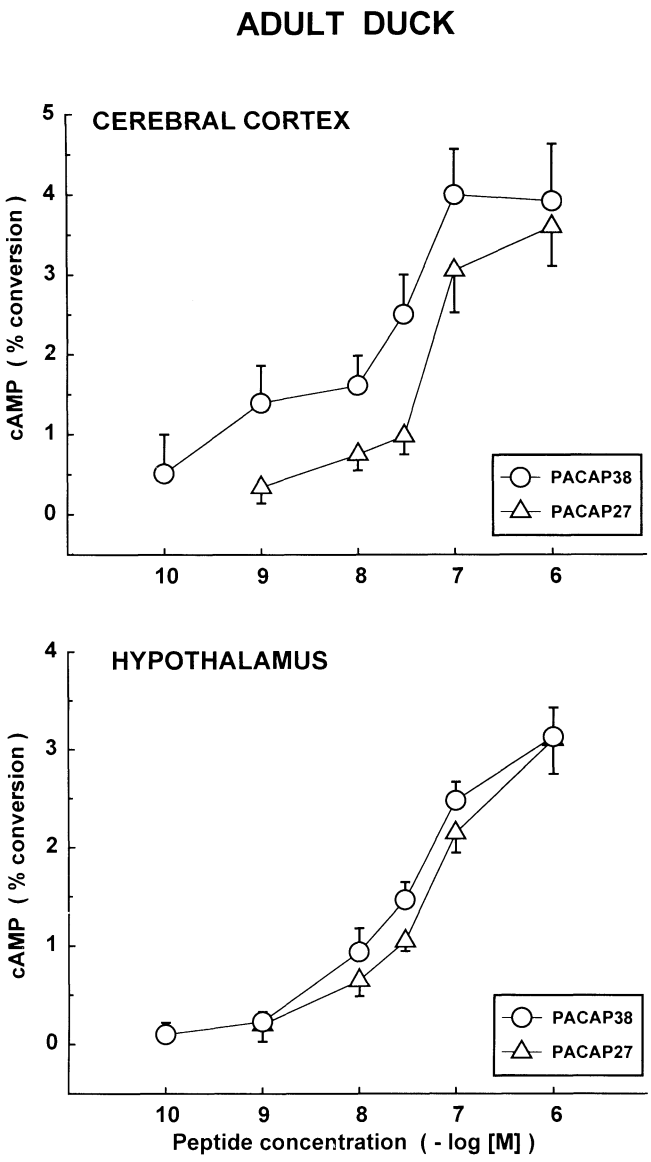

Fig. 2. The effects of $PACAP_{38}$ and $PACAP_{27}$ on cyclic AMP formation in [^{3}H]adenine-prelabeled slices of the cerebral cortex and hypothalamus of adult duck. Results are expressed in percent conversion and represent means ± SEM, showing "net" effects of the peptides, i.e., after subtraction of respective basal (control) values which were (in percent conversion): cerebral cortex, 1.98 ± 0.16(6) and 1.85 ± 0.21(8); hypothalamus, 0.89 ± 0.11(5) and 1.09 ± 0.15(10) for $PACAP_{38}$ (n = 4-9 for points on the curve) and $PACAP_{27}$ (8-10), respectively.

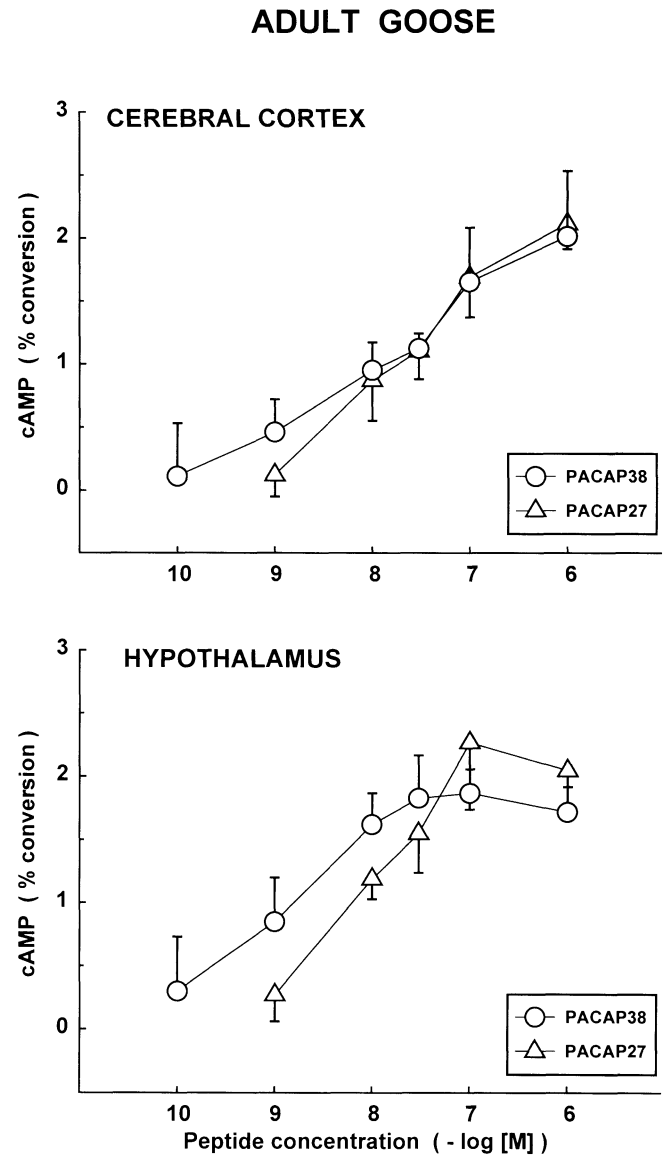

Fig. 3. The effects of $PACAP_{38}$ and $PACAP_{27}$ on cyclic AMP formation in [^{3}H]adenine-prelabeled slices of cerebral cortex and hypothalamus of adult goose. Results are expressed in per cent conversion and represent means ± SEM, showing "net" effects of the peptides, i.e., after subtraction of respective basal (control) values which were (in percent conversion): cerebral cortex, 1.49 ± 0.15(5) and 1.79 ± 0.10(8); hypothalamus, 0.85 ± 0.04(5) and 1.36 ± 0.09(9) for $PACAP_{38}$ (n = 4-5 for points on the curve) and $PACAP_{27}$ (n = 8-14), respectively.

tex). The observed rank-order of potency in evoking the cyclic AMP response in the chick brain, i.e. $PACAP_{38} \approx PACAP_{27} >> VIP$, indicates the role of PAC_1-subtype receptor in mediating the reported PACAP effects (Rawlings and Hezareh 1996, Harmar et al. 1998). In the duck CNS the difference in potency between VIP and $PACAP_{38}$ or $PACAP_{27}$ was of one order of magnitude (in chicks - three orders of magnitude; Nowak et al. 1999a,b), which may suggest that the receptor mediating the PACAP action on cyclic AMP production in ducks may also represent the PAC_1 subtype. It should be noted that the presence of PAC_1 receptors in the chick brain has already been identified by molecular biology approaches (Peeters et al. 1999). In addition to PAC_1 receptors, which prefer PACAP over VIP, these peptides can interact with other receptors known as $VPAC_1$ and $VPAC_2$ that are equally sensitive to both forms of PACAP and VIP ($PACAP_{38} \approx PACAP_{27} \approx VIP$) (Arimura and Shioda 1995, Rawlings and Hezareh 1996, Harmar et al. 1998).

Although it is not proven, the current results may suggest that in the CNS of the chick (Nowak et al. 1999a,b), duck (present data), and possibly goose, there may be a similar type of PACAP receptor, whose density (likely responsible for the peptide maximal effect) may differ among species. Another aspect worth of mentioning is that the difference in maximal responses observed in young and adult ducks and adult geese on one side, and 2-4-weeks-old chicks on the other side, may be species-, and not age- or way of breeding-dependent, as we did not detect a significant difference between the effects of PACAP between 2-3-weeks-old ducks (kept from the day of hatching under controlled daily light-dark cycle, room temperature and access to food) and adult animals grown under natural environmental conditions.

In terms of E_{max} and EC_{50} values characterizing the action of PACAP on cyclic AMP generation, and/or some differences in the effects observed between the two forms of the peptide, the presented here results, including those obtained for the chick CNS (Peeters et al. 1998b, Nowak et al. 1999a,b), are not unique for avians, as tremendous variability in the mentioned parameters has already been reported by many researchers. Such a variability appears to be dependent on a given tissue and species, as well as type and density of PACAP receptors. For example, in the rat pituitary cell cultures both forms of PACAP (27 and 38) showed a similar potency, with $E_{max} \approx 400\%$ of control reached at 0.01 μM (Miyata et al. 1990); both peptides showed even greater potency in the rat cerebellar neuroblasts, reaching E_{max} of about 1000% of control at low nanomolar concentrations (Basile et al. 1995). However, in the monkey retina, the maximal stimulation of adenylyl cyclase activity, produced by 0.01 μM of $PACAP_{38}$ and 0.1 μM of $PACAP_{27}$, reached only the value of 70% above basal level (Nilsson et al. 1996), whereas in the rat retina the reported E_{max} was about 800% of control, reached by $PACAP_{38}$ at 0.01 μM and $PACAP_{27}$ at a concentration >1 μM (Onali and Olianas 1994). In nonmammalian tissues, such as fragments of the frog anterior pituitary, the reported parameters for $PACAP_{38}$ were: $E_{max} \approx 1000\%$ of control, and $EC_{50} = 0.21$ μM (Chartel et al. 1991), while in the frog hypothalamic slices 3 μM of $PACAP_{27}$ increased cyclic AMP production by 168% above basal level, yet still not reaching a plateau (Wong et al. 1998).

In the vast literature regarding PACAP, we found only one paper describing distribution of PACAP-containing perikarya and nerve fibers in the avian CNS (Peeters et al. 1998a). This recently published study, carried out on chicken forebrain, showed a clearly larger density of the peptide-immunoreactive cells in the anterior part of the hypothalamus (preoptic and supraoptic region), in the nucleus paraventricularis magnocellularis, in the median eminence, as well as in the posterior lobe of pituitary. Assuming the existence of an analogy in the PACAP-specific neuronal network in brains among different avian species, the PACAP-containing cells (identified in chicken forebrain) may likely be a source of the endogenous peptide in the hypothalamus of the duck and goose. Yet, more study is needed to verify such a suggestion. Peeters et al. (1998b) have recently reported that PACAP is capable of stimulating growth hormone secretion from cultured chicken pituitary cells, suggesting that one of the peptide's physiological roles in chicks may be the role of a neuroendocrine regulator. If this holds true for ducks and geese, one might hypothesize on the neuroendocrine role of PACAP in avians generally.

In conclusion, this paper extends our earlier observations made on chicks, and shows that $PACAP_{27}$ and $PACAP_{38}$ are capable of potently stimulating cyclic AMP biosynthesis in the hypothalamus and cerebral cortex of ducks and geese, including both young animals grown under controlled conditions and adult animals grown "freely" in a natural environment.

We would like to thank Mrs. Teresa Kwapisz for her excellent technical assistance. This study was supported by the grant No 6P04C037-17 from the State Committee for Scientific Research (KBN) in Poland.

Arimura A., Shioda S. (1995) Pituitary adenylate cyclase activating polypeptide (PACAP) and its receptors: neuroendocrine and endocrine interactions. Front. Neuroendocrinol. 16: 53-88.

Basile M., Gonzalez B.J., Desrues L., Demas M., Fournier A., Vaudry H. (1995) Pituitary adenylate cyclase-activating polypeptide (PACAP) stimulates adenylyl cyclase and phospholipase C activity in rat cerebellar neuroblasts. J. Neurochem. 65: 1318-1324.

Chartrel N., Tonon M.C., Vaudry H., Conlon J.M. (1991) Primary structure of frog pituitary adenylate cyclase-activating polypeptide (PACAP) and effects of ovine PACAP on frog pituitary. Endocrinology 129: 3367-3371.

Christophe J. (1993) Type I receptors for PACAP (a neuropeptide even more important than VIP?). Biochem. Biophys. Acta 1154: 183-199.

Harmar A.J., Arimura A., Gozes I., Journot L., Laburthe M., Pisegna J.R., Rawlings S.R., Robberecht P., Said S.I., Sreedharan S.P., Wank S.A., Waschek J.A. (1998) International Union of Pharmacology. XVIII. Nomenclature of receptors for vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide. Pharmacol. Rev. 50: 265-270.

McRory J., Parker D.B., Ngamvongchon S., Sherwood N.M. (1995) Sequence and expression of cDNA for pituitary adenylate cyclase-activating peptide (PACAP) and growth hormone-releasing hormone (GHRH)-like peptide in catfish. Mol. Cell. Endocrinol. 108: 169-177.

Miyata A., Jiang L., Dahl R.D., Kitada C., Kubo K., Fujino M., Minamino N., Arimura A. (1990) Isolation of a neuropeptide corresponding to the N-terminal 27 residues of the pituitary adenylate cyclase activating polypeptide with 38 residues (PACAP38). Biochem. Biophys. Res. Commun. 170: 643-648.

Nilsson S.F.E., Amer N., Vandermeers A. (1996) Adenylate cyclase activation by VIP and PACAP in the retina and choroid: effects of antagonists. Ann. NY Acad. Sci. 805: 749-752.

Nowak J.Z., Kuba K., Zawilska J.B. (1999a) PACAP-induced formation of cyclic AMP in the chicken brain: regional variations and the effect of melatonin. Brain Res. 830: 195-199.

Nowak J.Z., Kuba K., Zawilska J.B. (1999b) Stimulatory effect of pituitary adenylate cyclase-activating polypeptide (PACAP) on cyclic AMP formation in the hypothalamus and cerebral cortex of four avians and rat. Pol. J. Pharmacol. 51: 87-91.

Nowak J.Z., Sek B. (1994) Stimulatory effect of histamine on cyclic AMP formation in chick pineal gland. J. Neurochem. 63: 1338-1345.

Nowak J.Z., Sek B., D'Souza T., Dryer S.E. (1995) Does histamine stimulate cyclic AMP formation in the avian pineal gland via a novel (non-H1, non-H2, non-H3) histamine receptor subtype. Neurochem. Int. 27: 519-526.

Onali P., Olianas M.C. (1994) PACAP is a potent and highly effective stimulator of adenylyl cyclase activity in the retinas of different mammalian species. Brain Res. 641: 132-134.

Peeters K., Berghman L.R., Vandesande F. (1998a) Comparative distribution of pituitary adenylate cyclase-activating polypeptide and vasoactive intestinal polypeptide immunoreactivity in the chicken forebrain. Ann. NY Acad. Sci. 839: 417-419.

Peeters K., Gerets H.H.J., Princen K., Vandesande F. (1999) Molecular cloning and expression of a chicken pituitary adenylate cyclase-activating polypeptide receptor. Mol. Brain Res. 71: 244-255.

Peeters K., Langouche L., Vandesande F., Darras V.M., Berghman L.R. (1998b) Effects of pituitary adenylate cyclase-activating polypeptide (PACAP) on cAMP formation and growth hormone release from chicken anterior pituitary cells. Ann. NY Acad. Sci. 865: 471-474.

Rawlings S.R., Hezareh M. (1996) Pituitary adenylate cyclase-activating polypeptide (PACAP) and PACAP/vasoactive intestinal polypeptide receptors: actions on the anterior pituitary gland. Endocrine Rev. 17: 4-29.

Salomon Y., Londos C., Rodbell M. (1974) A highly sensitive adenylate cyclase assay. Anal. Biochem. 58: 541-548.

Shimizu H., Daly J.W., Creveling C.R. (1969) A radioisotopic method for measuring the formation of adenosine 3',5'-cyclic monophosphate in incubated slices of brain. J. Neurochem. 16: 1609-1619.

Wong A.O.L., Leung M.Y., Shea W.L.C., Tse L.Y., Chang J.P., Chow B.K.C. (1998) Hypophysiotropic action of pituitary adenylate cyclase-activating polypeptide (PACAP) in the goldfish: immunohistochemical demonstration of PACAP in the pituitary, PACAP stimulation of growth hormone release from pituitary cells, and molecular cloning of pituitary type I PACAP receptor. Endocrinology 139: 3465-3479.

Yon L., Feuilloley M., Chartrel N., Arimura A., Conlon J.M., Fournier A., Vaudry H. (1992) Immunohistochemical distribution and biological activity of pituitary adenylate cyclase-activating polypeptide (PACAP) in the central nervous system of the frog *Rana ridibunda*. J. Comp. Neurol. 324: 485-499.

Received 20 December 1999, accepted 7 February 2000