Proadrenomedullin N-terminal 20 peptide (PAMP) elevates blood glucose levels via bombesin receptor in mice

Kousaku Ohinata[a], Akio Inui[b], Akihiro Asakawa[b], Keiji Wada[c], Estuko Wada[c], Masaaki Yoshikawa[a,*]

[a] *Department of Functional Food Resources, Research Institute for Food Science, Kyoto University, Gokasho Uji, Kyoto 611-0011, Japan*
[b] *Second Department of Internal Medicine, Kobe University School of Medicine, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan*
[c] *Department of Degenerative Neurological Diseases, National Institute of Neuroscience, NCNP 4-1-1 Ogawahigashi, Kodaira, Tokyo 187-8502, Japan*

Received 21 March 2000; received in revised form 10 April 2000

Edited by Shozo Yamamoto

Abstract **We found a potent hyperglycemic effect of proadrenomedullin N-terminal 20 peptide (PAMP) after intra-third cerebroventricular administration at a dose of 10 nmol in fasted mice. PAMP has four homologous residues with bombesin (BN), a hyperglycemic peptide. PAMP showed affinity for gastrin-releasing peptide preferring receptor (GRP-R) and neuromedin B preferring receptor. The PAMP-induced hyperglycemic effect was inhibited by [D-Phe^6, Leu-NHEt^{13}, des-Met^{14}]-BN (6–14), GRP-R specific antagonist, indicating that the hyperglycemic effect is mediated at least in part via GRP-R. Furthermore, pretreatment of α-adrenergic blocker inhibited the PAMP-induced hyperglycemia and hyperglucagonemia, suggesting that the increase of glucagon secretion through α-adrenergic activation is involved in this hyperglycemic effect of PAMP.**

Key words: Proadrenomedullin N-terminal 20 peptide; Hyperglycemia; Bombesin; Gastrin-releasing peptide preferring receptor; α-Adrenergic activation

1. Introduction

Proadrenomedullin N-terminal 20 peptide (PAMP) is a bioactive peptide which is derived from a precursor of potent hypotensive peptide adrenomedullin (AM) originally isolated from the human pheochromocytoma [1]. The AM gene is transcribed and the gene product translates within not only peripheral organs such as the adrenal gland and vasoculature but also in the central nervous system [2]. AM is a novel member of the calcitonin gene-related peptide (CGRP) superfamily, and has affinity for CGRP receptor and AM receptor. AM has been shown to mediate, at least in part, the vasodilator effect through the CGRP receptor. Peripherally administered PAMP transiently lowers blood pressure, although its effect is less potent than AM [3]. Except for its hypotensive effect, the biological function of PAMP presently remains unclear. It was reported using a radioligand of PAMP that specific binding sites for PAMP are widely distributed in plasma and tissues such as the brain, lung and kidney, and relatively abundant binding sites are present in the adrenal glands and aorta [4]. Despite much effort, however, the PAMP receptor has not been cloned.

We found that centrally administered PAMP potently and rapidly elevates blood glucose levels in fasted mice. In a previous study, we reported that bioactive peptides derived from food protein, with only three or four homologous residues with the endogenous bioactive peptides, have affinity for their receptors [5–7]. We noticed that PAMP (ARLDVASEFRKKWNKWALSR-NH_2) has four homologous residues with bombesin (BN) (Pyr-QRLGNQWAVGHLM-NH_2), which exerts hyperglycemia after central administration [8,9].

BN is an amidated tetradecapeptide first isolated from amphibian skin [10]. BN-like peptides (gastrin-releasing peptide [11], neuromedin B [12] and neuromedin C [13]) have also been demonstrated to exist throughout the mammalian gastrointestinal and nervous system. The three known mammalian BN receptor subtypes are the gastrin-releasing peptide preferring receptor (GRP-R) [14], neuromedin B preferring receptor (NMB-R) [15] and BN receptor subtype 3 (BRS-3) [16]. Exogenous administration of these BN-like peptides into various organ systems elicits a wide range of responses including secretion of gastrointestinal hormones and gastric acid, and regulation of smooth muscle [17]. These peptides are postulated to play roles in thermoregulation, metabolism, behavior and satiety.

In the present study, we investigated whether PAMP has affinity for the BN receptor. [D-Phe^6, Leu-NHEt^{13}, des-Met^{14}]-BN (6–14) [18,19] was used to examine whether the hyperglycemic effect is mediated via GRP-R receptor. Furthermore, we evaluated the involvement of the sympathetic nervous system in the action of PAMP, using phentolamine (α-adrenergic blocker).

2. Materials and methods

2.1. Materials

Human PAMP, PAMP-related peptide and [D-Phe^6, βAla^{11}, Phe^{13}, Nle^{14}]-BN (6–14) were synthesized by Fmoc strategy using a PS3 peptide synthesizer (Protein Technologies, USA). The peptides were purified by reverse-phase high performance liquid chromatography on an octadecyl silica column. BN, neuromedin B and neuromedin C were purchased from the Peptide Institute Inc. (Osaka, Japan). ^{125}I-[Tyr^4]-BN was obtained from NEN (Boston, MA, USA). BALB 3T3 cells stably transfected with NMB-R, GRP-R, BRS-3 were a gift from Mitsubishi-Tokyo Pharmaceuticals Inc. (Tokyo, Japan). Dulbecco's modified Eagle medium (DMEM) and G418 sulfate (geneticin) were obtained from Gibco BRL (Grand Island, NY, USA). The 0.05% trypsin–EDTA solution, soybean trypsin inhibitor, benzamidine, bovine serum albumin and phentolamine were from Sigma Chemical (St.

*Corresponding author. Fax: (81)-774-38 3774.
E-mail: yosikawa@food2.food.kyoto-u.ac.jp

Abbreviations: PAMP, proadrenomedullin N-terminal 20 peptide; BN, bombesin; GRP-R, gastrin-releasing peptide preferring receptor; i.c.v., intra-third cerebroventricular

PII: S0014-5793(00)01529-5

Louis, MO, USA). Bacitracin was purchased from Wako (Osaka, Japan). GRP-R antagonist, [D-Phe^6, Leu-NHEt^{13}, des-Met^{14}]-BN (6–14) was obtained from Bachem (Bubendorf, Switzerland).

2.2. Animals

Male ddY mice (SLC, Shizuoka, Japan) at 7 weeks of age served as the experimental animals. Each mouse was individually housed in regulated conditions (22°C on a 12 h light–dark cycle). Food and water were available ad libitum, except as otherwise indicated.

Intra-third cerebroventricular (i.c.v.) injection was performed as described previously [20]. Mice were anesthetized with sodium pentobarbital (80–85 mg/kg intraperitoneal (i.p.)) and placed in a stereotaxic instrument. A hole was made through the skull using a needle aimed 0.9 mm lateral to the suture and 0.9 mm posterior to the bregma. A 24-gauge cannula beveled at one end over a 3 mm distance (Safelet-Cas, Nipro, Osaka, Japan) was implanted into the third cerebral ventricle. Animals were tested 1 week or more after implantation.

2.3. Effect of PAMP on blood glucose

Mice were deprived of food for 18 h with free access to water. Blood samples were obtained from the orbital sinus under ether anesthesia at 15, 30 and 60 min after i.c.v. injection of PAMP in 4 μl of artificial cerebrospinal fluid (ACSF) or ACSF alone. Plasma was separated from the blood and stored at −20°C until analysis. All experiments were approved by our University animal committee.

2.4. BN antagonist and α-adrenergic blocker studies

To establish whether the effect of central administered PAMP was via GRP-R, the blood glucose was measured at 15 min after i.c.v. injection of 10 nmol PAMP and [D-Phe^6, Leu-NHEt^{13}, des-Met^{14}]-BN (6–14), BN antagonist. To understand the underlying mechanism for the rapid hyperglycemic response to PAMP, 10 mg/kg phentolamine or saline was peripherally injected 30 min before i.c.v. injection of PAMP or ACSF. The blood glucose and plasma glucagon levels were determined 15 min after i.c.v. administration.

2.5. Biochemical analyses

Blood glucose was determined by the glucose oxidase method. NEFAs, triglycerides and cholesterols were measured with an enzymatic colorimetric assay (Wako, Osaka, Japan). Insulin levels were determined using an enzyme-linked immunosorbent assay insulin kit (Morinaga, Tokyo, Japan). Glucagon and corticosterone levels were assayed by RIA.

2.6. Growth and maintenance of cells

BALB 3T3 cells stably transfected with NMB-R, GRP-R and BRS-3 were grown in DMEM containing 25 mM HEPES. All cell media were supplemented with 10% (v/v) fetal bovine serum (JRH biosciences), 25 IU/ml penicillin (Banyu Pharmaceutical Co., Ltd., Tokyo, Japan), 25 μg/ml streptomycin (Meiji Seika Kaisha, Ltd., Tokyo, Japan), 1.25 μg/ml amphotericin (ICN Pharmaceuticals, Inc., Costa Mesa, CA, USA) and 400 μg/ml geneticin. All cells were maintained at 37°C in a 5% CO_2 atmosphere. Cells were subcultured every 3–4 days at confluence after detaching the cells with trypsin–EDTA solution.

2.7. Preparation of membrane from cells

The homogenizing buffer [21] contained 50 mM Tris–HCl (pH 7.4), 0.2 mg/ml soybean trypsin inhibitor, 0.2 mg/ml benzamidine and 0.1% bacitracin. Then 1×10^7 cells/ml were homogenized at 4°C with Biomixer (Nihonseiki Kaisha Ltd.) for 30 s. The homogenized suspension was centrifuged at 1500 rpm for 10 min at 4°C. The supernatant was removed and recentrifuged at 20 000 rpm for 20 min. The pellet was resuspended in homogenizing buffer and stored at −80°C.

2.8. BN receptor assay

The radioreceptor assay was performed according to the method of Moody et al. [22]. Briefly, 50 pM ^{125}I-[Tyr^4]-BN was incubated with the membrane from rat brain or cells stably transfected with NMB-R, GRP-R and BRS-3 at 4°C for 24 min in 50 mM Tris–HCl buffer containing 2 μg/ml bacitracin and 0.1% bovine serum albumin (w/v) (pH 7.4). Membrane bound ^{125}I-[Tyr^4]-BN was separated by filtration through a GF/B filter (Whatman, Maidstone, UK). The filter was washed promptly with ice cold 50 mM Tris–HCl buffer containing 0.1% bovine serum albumin and assayed for radioactivity in a gamma counter. Specific binding was obtained by subtracting non-specific binding in the presence of an excess (100 μM) unlabeled BN from total binding.

2.9. Statistical analysis

Values are expressed as the mean ± S.E.M. Individual group means were compared by either unpaired Student's t test for two-group comparisons or multiple Bonferroni comparison for more than two groups. P values of less than 0.05 were considered to be statistically significant.

3. Results

The effects of i.c.v. administration of PAMP on blood glucose are shown in Fig. 1. The initial experiment showed that PAMP (10–30 nmol/mouse) potently increased blood glucose levels at 30 min after central injection in a dose-dependent manner (Fig. 1A). In the time course study, 10 nmol of PAMP potently and rapidly increased blood glucose levels at 15 min after i.c.v. injection, and the hyperglycemia lasted for 1 h (Fig. 1B).

Due to the sequence homology of PAMP with BN, the receptor affinity of PAMP for the BN receptor was investigated. In the rat brain membrane, 50% of specific ^{125}I-[Tyr^4]-BN binding (IC_{50}) was inhibited when the BN concentration was 5.0×10^{-9} M. PAMP had affinity for the BN receptor ($IC_{50} = 5.2\times10^{-8}$ M) (Table 1), although it was one tenth lower than that of BN. The affinities of PAMP(1–20)-OH and PAMP(9–20)-NH_2 for BN receptor were lower ($IC_{50} = 2.2\times10^{-7}$ M) than PAMP. The relative potency of the BN receptor affinity was PAMP > PAMP(1–20)-OH = PAMP(9–20)-NH_2 > PAMP(12–20)-NH_2 > PAMP(13–20)-N-

Table 1
The affinities of PAMP, PAMP-related peptides and BN-related peptides for BN receptor in rat brain membrane or cells stably transfected with NMB-R, GRP-R and BRS-3 (IC_{50}; M)

Peptide	Brain membrane	Cells stably transfected with BN receptor			
		rNMB-R	mGRP-R	hBRS-3	mBRS-3
PAMP	5.2×10^{-8}	1.8×10^{-8}	2.2×10^{-8}	6.8×10^{-8}	6.8×10^{-8}
BN	5.0×10^{-9}	1.4×10^{-8}	3.3×10^{-9}	3.3×10^{-9}	3.3×10^{-9}
Neuromedin C	5.4×10^{-9}	4.0×10^{-8}	5.0×10^{-9}	7.8×10^{-9}	5.3×10^{-9}
Neuromedin B	2.4×10^{-8}	1.3×10^{-9}	2.6×10^{-8}	4.7×10^{-8}	4.3×10^{-8}
[D-Phe, βAla^{11}, Phe^{13}, Nle^{14}]-BN (6–14)	1.0×10^{-9}	1.5×10^{-8}	1.3×10^{-9}	1.4×10^{-9}	1.4×10^{-9}
PAMP(1–20)-OH	2.2×10^{-7}				
PAMP(9–20)-NH_2	2.2×10^{-7}				
PAMP(12–20)-NH_2	9.1×10^{-7}				
PAMP(13–20)-NH_2	1.7×10^{-6}				
PAMP(13–20)-OH	1.6×10^{-5}				

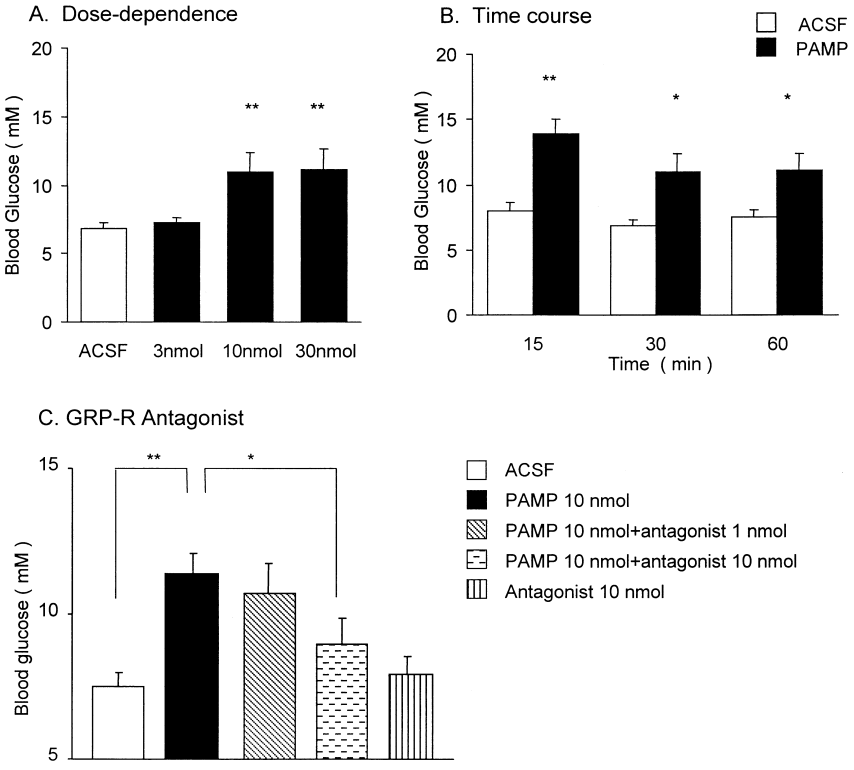

Fig. 1. Effect of i.c.v. administration of PAMP on blood glucose in fasted mice. Male ddY mice were injected with 4 μl of 3, 10 or 30 nmol PAMP, and the blood glucose was measured 30 min after central administration (A). In the time course study (B), 10 nmol of PAMP was i.c.v. administered. In GRP-R antagonist study (C), 10 nmol PAMP and 1 or 10 nmol [D-Phe^{6}, Leu-NHEt^{13}, des-Met^{14}]-BN (6–14) were i.c.v. coadministered, and the blood glucose was measured 15 min after administration. Each column represents the mean ± S.E.M. of 5–7 mice. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the ACSF-treated group using the unpaired Student's t test in A and B, and compared with each group by Bonferroni's t test in C.

H_2 > PAMP(13–20)-OH. From these findings, it was indicated that the amide group and the amino acid sequence at the C-terminal of PAMP were necessary to bind the BN receptor.

To compare the affinities of PAMP for each BN receptor subtype, BALB 3T3 cells stably transfected with NMB-R, GRP-R and BRS-3 were used. PAMP had high affinities for NMB-R and GRP-R; the IC_{50} values were 1.8×10^{-8} and 2.2×10^{-8} M, respectively (Table 1). The affinity of PAMP for BRS-3 was less potent than NMB-R and GRP-R ($IC_{50} = 6.8 \times 10^{-8}$ M).

To investigate whether PAMP elevates blood glucose via the BN receptor, we coadministered PAMP and GRP-R antagonist, [D-Phe^{6}, Leu-NHEt^{13}, des-Met^{14}]-BN (6–14). The increase of blood glucose after central administration of 10 nmol of PAMP was 63% inhibited by 10 nmol of GRP-R antagonist (Fig. 1C). The dose of the GRP-R antagonist was limited to the solubility of that in ACSF.

Table 2 shows the effect of PAMP on plasma insulin, NEFA, triglyceride and cholesterol levels. There was no difference in the insulin levels at 15 min after i.c.v. injection. The plasma insulin levels were significantly increased at 60 min after i.c.v. administration of PAMP, following the hyperglycemic action of PAMP. The NEFA levels at 30 min after PAMP administration were slightly higher than ACSF. There was no difference in the concentrations of plasma triglycerides and cholesterol after injection of PAMP in the present study.

The effects of phentolamine, α-adrenergic blocker, on PAMP-induced hyperglycemia are shown in Fig. 2A. First, 10 mg/kg phentolamine or saline was peripherally injected 30 min before i.c.v. administration of PAMP or ACSF. The i.p. administered phentolamine lowered the basal blood glucose levels in mice injected with ACSF. The phentolamine pretreatment inhibited the PAMP-induced hyperglycemia by 50% at 15 min after i.c.v. injection, with the effect being highly significant. Glucagon levels were significantly elevated at 15 min post i.c.v. administration of PAMP, and the pretreatment

Table 2
Effect of i.c.v. injection of PAMP (10 nmol/mouse) on plasma insulin, NEFA, triglyceride and cholesterol concentrations in fasted mice

Time after injection	15 min		30 min		60 min	
Group	ACSF	PAMP	ACSF	PAMP	ACSF	PAMP
Insulin (pM)	63.5 ± 28.6	50.1 ± 22.9	68.7 ± 13.5	120.9 ± 29.6	58.9 ± 9.8	109.3 ± 21.7*
NEFA (g/l)	0.15 ± 0.01	0.15 ± 0.01	0.24 ± 0.01	0.27 ± 0.01*	0.13 ± 0.01	0.15 ± 0.01
Triglyceride (g/l)	0.61 ± 0.12	0.53 ± 0.07	0.66 ± 0.04	0.55 ± 0.08	0.62 ± 0.10	0.45 ± 0.05
Cholesterol (mM)	3.86 ± 0.52	3.79 ± 0.33	3.49 ± 0.14	3.73 ± 0.26	3.11 ± 0.34	3.13 ± 0.28

Values are expressed as mean ± S.E.M. of 6–7 mice.
$^{*}P < 0.05$ compared with the ACSF-treated group by Student's t test.

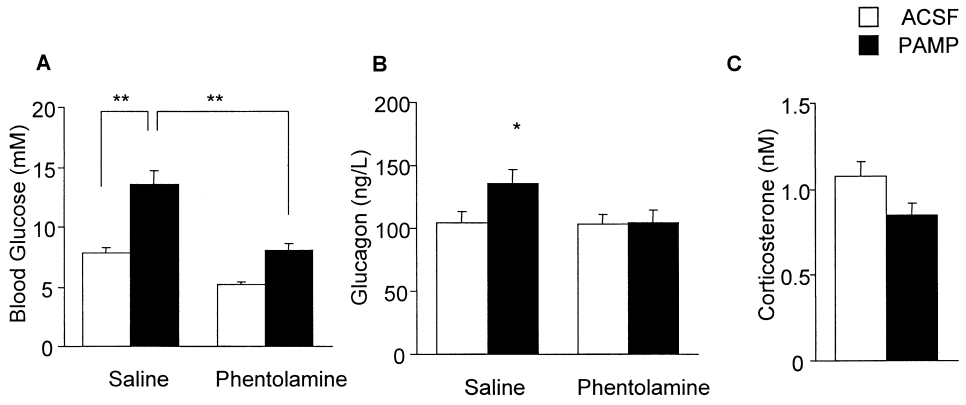

Fig. 2. Effect of α-adrenergic blocker on PAMP-induced elevations of blood glucose (A) and glucagon levels (B) in mice ($n = 7$–8). Phentolamine (10 mg/kg i.p.) or saline was injected 30 min before i.c.v. injection of PAMP or ACSF. The blood glucose and plasma glucagon levels were determined 15 min after i.c.v. administration. Corticosterone levels (C) were taken at 15 min after i.c.v. injection of PAMP in mice ($n = 6$). Each column represents mean ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the ACSF-treated group or saline-pretreated group by Bonferroni's t test.

of α-blocker effectively abolished the hyperglucagonemic response (Fig. 2B). There was no difference in plasma corticosterone levels between the control and ACSF-treated animals at 15 min after i.c.v. administration (Fig. 2C).

4. Discussion

We have shown, for the first time, that centrally administered PAMP potently and rapidly increases blood glucose in fasted conscious mice in a dose-dependent manner. The findings of the binding assay indicated that PAMP had high affinity for GRP-R and NMB-R. The PAMP-induced hyperglycemic effect was significantly reversed by GRP-R antagonist, [D-Phe^{6}, Leu-NHEt^{13}, des-Met^{14}]-BN (6–14), suggesting that the hyperglycemic effect was, at least in part, mediated via GRP-R.

Centrally administered PAMP acutely exerted a marked hyperglycemic action in conscious animals within 15 min. There was no difference in the plasma insulin levels between ACSF- and PAMP-treated animals 15 min after administration, although the insulin levels increased thereafter. The subsequent hyperinsulinemia appeared to be secondary to the acute hyperglycemia.

To understand the underlying mechanism for the rapid hyperglycemic response to centrally administered PAMP, the effects of peripherally pretreated α-adrenergic blocker (phentolamine) on PAMP-induced hyperglycemia and plasma glucagon levels were investigated in mice. The i.p. pretreatment of α-adrenergic blocker inhibited the hyperglycemic response to i.c.v. PAMP by 50%. It is well-known that increased glucagon secretion, in concert with adrenergic mechanisms, is a primary counter-regulatory response for the recovery of glucose levels during hypoglycemia [23,24]. Central administration of PAMP stimulated the glucagon secretion and the PAMP-induced hyperglucagonemia was inhibited by the pretreatment with α-blocker. Furthermore, peripheral pretreatment with pentobarbital, which suppresses the activation of the sympathetic nervous system [25], effectively inhibited the PAMP-induced hyperglycemic response in the preliminary experiment (data not shown). On the other hand, there was no difference in plasma corticosterone levels between controls and PAMP-treated mice. These findings suggest that PAMP-induced hyperglycemia after i.c.v. administration is due, at least in part, to the increased glucagon secretion, through α-adrenergic activation in the sympathetic nervous system.

The plasma NEFA levels were assayed, because the release of glucose and NEFA from their storage sites in the liver and white adipose tissue, respectively, are controlled by separable central systems with different peripheral mechanisms [26]. The plasma NEFAs at 30 min post i.c.v. injection of PAMP were slightly but significantly higher than ACSF. Glucagon secretion may participate in the NEFA release after central administration of PAMP.

Both PAMP [27] and BN [28] exert hypertensive actions when administered centrally in conscious rats. These effects are mediated via activation of the sympathetic α-adrenergic nerve system. The hypertensive effect of centrally injected PAMP may thus be mediated via BN receptor. However, it has been reported that peripheral administration of PAMP decreases blood pressure in conscious rats [29] and BN inversely exhibits the hypertensive action [30]. This may suggest the existence of a more specific PAMP receptor. Further investigations may provide additional insight into the role of PAMP in the cardiovascular system.

In conclusion, the present findings demonstrate that centrally administered PAMP elevates blood glucose and PAMP has affinity for the BN receptor. The PAMP-induced hyperglycemic effect was inhibited by GRP-R antagonist, suggesting that PAMP action is mediated, at least in part, through GRP-R. Furthermore, the hyperglycemia involves the increase of glucagon secretion through α-adrenergic activation.

Acknowledgements: This work was supported in part by grants from the Ministry of Education, Science, Sports, and Culture of Japan to M.Y. and A.I., and PROBRAIN grant from Bio-oriented Technology Research Advancement Institution to M.Y. We thank Mitsubishi-Tokyo Pharmaceuticals Inc. for BALB 3T3 cells stably transfected with NMB-R, GRP-R and BRS-3 in BN binding assay.

References

[1] Kitamura, K., Sakata, J., Kangawa, K., Kojima, M., Matsuo, H. and Eto, T. (1993) Biochem. Biophys. Res. Commun. 194, 720–725.
[2] Takahashi, K., Satoh, F., Sone, M., Murakami, O., Sasano, H., Mouri, T. and Shibahara, S.. (1997) Peptides 18, 1051–1103.
[3] Kitamura, K., Kangawa, K., Ishiyama, Y., Washimine, H., Ichi-

ki, Y., Kawamoto, M., Minamino, N., Matsuo, H. and Eto, T. (1994) FEBS Lett. 351, 35–37.
[4] Iwasaki, H., Hirata, Y., Iwashina, M., Sato, K. and Marumo, F. (1996) 137, 3045–3050.
[5] Fujita, H., Usui, H., Kurahashi, K. and Yoshikawa, M. (1995) Peptides 16, 785–790.
[6] Takahashi, M., Moriguchi, S., Ikeno, M., Kono, S., Ohata, K., Usui, H., Kurahashi, K., Sasaki, R. and Yoshikawa, M. (1996) Peptides 17, 5–12.
[7] Fukudome, S. and Yoshikawa, M. (1992) FEBS Lett. 296, 107–111.
[8] Iguchi, A., Matsunaga, H., Nomura, T., Gotoh, M. and Sakamoto, N. (1984) Endocrinology 114, 2242–2246.
[9] Gotoh, M., Iguchi, A., Yanase, M., Futenma, A., Nakamura, S., Kato, K. and Sakamoto, N. (1988) Endocrinol. Jpn. 35, 639–645.
[10] Anastasi, A., Erspamer, V. and Bucci, M. (1971) Experientia 27, 166–167.
[11] McDonald, T.J., Jornvall, H., Nilsson, G., Vagne, M., Ghatei, M., Bloom, S.R. and Mutt, V. (1979) Biochem. Biophys. Res. Commun. 90, 227–233.
[12] Minamino, N., Kangawa, K. and Matsuo, H. (1983) Biochem. Biophys. Res. Commun. 114, 541–548.
[13] Minamino, N., Kangawa, K. and Matsuo, H. (1984) Biochem. Biophys. Res. Commun. 119, 14–20.
[14] Spindel, E.R., Giladi, E., Brehm, P., Goodman, R.H. and Segerson, T.P. (1990) Mol. Endocrinol. 4, 1956–1963.
[15] Wada, E., Way, J., Shapira, H., Kusano, K., Lebacq-Verheyden, A.M., Coy, D., Jensen, R. and Battery, J. (1991) Neuron 6, 421–430.
[16] Gorbulev, V., Akhundova, A., Buchner, H. and Fahrenholz, F. (1992) Eur. J. Biochem. 208, 405–410.
[17] Lebacq-Verheyden A.M., Trepel, J., Sausville, E.A. and Battey, J.F. (1990) Innerarity, in: Handbook of Experimental Pharmacology (Sporn, M. and Roberts, A., Eds.), Vol. 95, pp. 71–142, Springer, Berlin.
[18] von Schrenck, T., Wang, L.H., Coy, D.H., Villanueva, M.L., Mantey, S. and Jensen, R.T. (1990) Am. J. Physiol. 259, G468–G473.
[19] Lin, J.T., Coy, D.H., Mantey, S.A. and Jensen, R.T. (1995) J. Pharmacol. Exp. Ther. 275, 285–295.
[20] Nakajima, M., Inui, A., Teranishi, A., Miura, M., Hirosue, Y., Okita, M., Himori, N., Baba, S. and Kasuga, M. (1994) J. Pharmacol. Exp. Ther. 268, 1010–1014.
[21] Mantey, S.A., Weber, H.C., Sainz, E., Akeson, M., Ryan, R.R., Pradhan, T.K., Searles, R.P., Spindel, E.R., Battey, J.F., Coy, D.H. and Jensen, R.T. (1997) J. Biol. Chem. 272, 26062–26071.
[22] Moody, T.W., Pert, C.B., Rivier, J. and Brown, M.R. (1978) Proc. Natl. Acad. Sci. USA 75, 5372–5376.
[23] Havel, P.J. and Valverde, C. (1996) Diabetes 45, 960–966.
[24] Cryer, P.E. (1993) Am. J. Physiol. 264, E149–E155.
[25] Smythe, G.A. and Edwards, S.R. (1992) Am. J. Physiol. 263, E823–E827.
[26] Gunion, M.W., Rosenthal, M.J., Miller, S., Hoyt, M.A. and Yonzon, D.B. (1992) Brain Res. 588, 84–96.
[27] Samson, W.K., Murphy, T.C. and Resch, Z.T. (1998) Am. J. Physiol. 274, R1505–R1509.
[28] Fisher, L.A., Cave, C.R. and Brown, M.R. (1985) Am. J. Physiol. 248, H425–H431.
[29] Saita, M., Ishizuka, Y., Kato, K., Kunitake, T., Hanamori, T., Kitamura, K., Eto, T. and Kannan, H. (1998) Regul. Pept. 77, 147–153.
[30] Bayorh, M.A. and Feuerstein, G. (1985) Peptides 6, 115–120.