Antidiuretic activities of substance P and its analogs[1]

M. Ukai, T. Nagase, M. Hirohashi and N. Yanaihara

Institute of Germfree Life Research, Nagoya University School of Medicine, Nagoya 466, and Shizuoka College of Pharmacy, Shizuoka 422 (Japan), 21 July 1980

Summary. Substance P (SP) and analogs, including 5 nucleoside (ARA or HRA)-peptides, were examined for antidiuretic activity in ethanolized rats. The activity was potent in the analogs embodying the C-terminal hexapeptide, weak in the nucleoside-pentapeptide, and negligible in the nucleoside-tetrapeptide. In addition, the activity was increased by acylation of the hexapeptide. The antidiuretic potencies were also compared with the hypotensive potencies.

The undecapeptide substance P (SP)[2-4], having the structure

1 2 3 4 5 6 7 8 9 10 11
H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2,

is known to possess a variety of biological properties. These include the gut- or gallbladder-contracting[2,4,5], hypotensive[3,4] sialogogic[4,6], glucagon-releasing[7,8], natriuretic[9], analgesic[10] and motoneuron-depolarizing[11] effects. Some of these effects were found to be affected in their potency by shortening the peptide length or replacing a single amino acid with another in the SP structure[12-14]. These results are in close agreement, leading to the assumption that the C-terminal part of the molecule may play an important role in determining the potency. This is supported by other studies using natural analogs of SP[15,16]. In most studies, however, the gut-contracting property has been used to evaluate the potency.

In the present work to elucidate the structure-activity relationship, the antidiuretic activities of SP and its analogs were examined in the rat, and compared with their hypotensive activities. A part of this study has been presented preliminarily[17].

Materials and methods. The synthetic SP and the 6 analogs used in this study are listed in the table. All the compounds were synthesized in Yanaihara's laboratory. The method for preparation of SP and its fragments have previously been described[13]. The nucleoside peptides were prepared by the coupling of the peptides with the protected nucleoside, 2,3-o-isopropylidene protected 1-(9-adenyl)-β-D-ribofuranuronic acid (ARA-OH) or 1-(9-hypoxanthyl)-β-D-ribofuranuronic acid (HRA-OH), which was followed by acidolysis[18] (figure 1). The details of the synthesis will be described elsewhere.

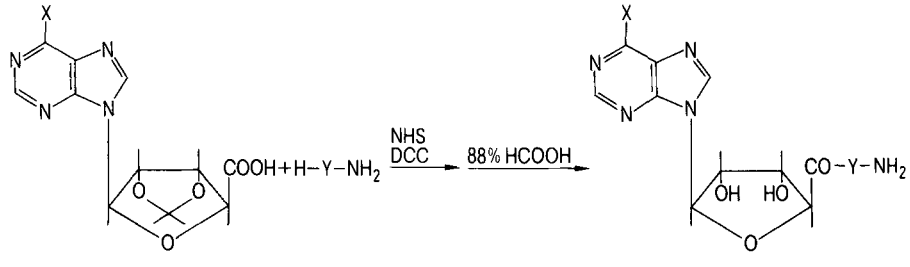
The antidiuretic activity was assayed in ethanolized male Sprague-Dawley rats[19,20] in which urine conductance and urine flow were continuously monitored, using the apparatus which was developed in Ukai's laboratory[21]. The responses to injections of SP or analogs dissolved in 0.2 ml of 'injection solution'[20] ($10^{-7}-10^{-4}$M) into a femoral vein were evaluated on the logarithm of the maximum conductance (C_{max}) divided by the initial conductance (C_{int}) or by the percent change in the areas under the curves of urine flow (figure 2). A 3-point assay design was used for antidiuretic activity, analogs being bracketed between 2 SP standards.

The hypotensive activity was examined in rats ethanolized in the same manner as was done for the antidiuretic activity. The apparatus described above was also available for recording the blood pressure of the carotid artery by means of a pressure transducer.

Results and discussion. Intravenous injection of SP caused a transient decrease in urine flow and a transient increase in urine conductance (figure 2). The maximal effects, which were distinguishable from those occurring 7 min after i.v. injection of vasopressin were observed 1 min after SP injection.

The relationship between the log dose of SP and the increase in log urine conductance was linear, indicating that the index of precision (λ) for the regression line was 0.17, while that between the log dose of SP and the % decrease in urine volume was also linear ($r=0.89$, $p<0.001$, $\lambda=0.24$) over the range of 9-200 pmoles of SP per rat weighing about 350 g. Thus we have established an assay system for evaluating the antidiuretic activity of SP on the increase in log urine conductance.

The antidiuretic activities of SP and analogs are shown in the table. When the activity of SP was defined as 100%, the ARA-hexapeptide, ARA-pentapeptide and ARA-tetrapeptide showed 53.9%, 3.5% and 0.1%, respectively. These data are compatible with the studies[13,22,23] indicating that the C-terminal hexapeptide is the active fragment of SP. Replacement of Phe with Ile at position 8 caused no significant change in antidiuretic activity. This is supported by the study which showed similar effects on other activities[24]. The introduction of the HRA group at the N-terminal of [Ile^8]SP_{6-11} increased the antidiuretic activity remarkably. An additional significant increase of the activity was achieved by exchanging HRA with ARA. This might be related to the polar groups (NH_2 in ARA, OH in HRA) in



Protected nucleoside Peptide Nucleoside peptide (A,B,C,E,F)

Fig. 1. Synthesis of nucleoside peptides, using dicyclohexylcarbodiimide (DCC) under the presence of N-hydroxysuccinimide (NHS).

Analog	X	Y
A	NH_2	GlnPhePheGlyLeuMet
B	NH_2	GlnPheIleGlyLeuMet
C	OH	GlnPheIleGlyLeuMet
E	NH_2	PhePheGlyLeuMet
F	NH_2	PheGlyLeuMet

Relative potencies of SP and its analogs in causing antidiuretic and hypotensive effects[a]

Analog	Chemical structure	Antidiuretic	Hypotensive
	SP	100	100
A	ARA-GlnPhePheGlyLeuMet-NH_2 (ARA-SP_{6-11})	53.9 ± 6.9 (4)[b]	142.7 ± 25.9 (6)
B	ARA-GlnPheIleGlyLeuMet-NH_2 (ARA-[Ile^8]SP_{6-11})	70.5 ± 4.2 (4)	9.2 ± 0.7 (6)
C	HRA-GlnPheIleGlyLeuMet-NH_2 (HRA-[Ile^8]SP_{6-11})	32.0 ± 5.3 (4)	6.7 ± 0.8 (6)
D	H-GlnPheIleGlyLeuMet-NH_2 ([Ile^8]SP_{6-11})	1.2 ± 0.3 (5)	0.9 ± 0.1 (6)
E	ARA-PhePheGlyLeuMet-NH_2 (ARA-SP_{7-11})	3.5 ± 0.3 (5)	4.1 ± 0.4 (6)
F	ARA-PheGlyLeuMet-NH_2 (ARA-SP_{8-11})	0.1 ± 0.0 (5)	0.4 ± 0.2 (6)

[a] Antidiuretic and hypotensive effects were evaluated on the increase in log urine conductance and on the decrease in carotid arterial pressure in response to SP and analogs, respectively. [b] Values are expressed as the mean ± SE (number of rats used), compared to the potency of SP (= 100). The potency order was SP > B ≧ A > C > E > D > F in antidiuretic effect and A ≧ SP > B > C > E > D > F in hypotensive effect, while significant ($p < 0.05$) and no significant differences are shown by > and ≧, respectively.

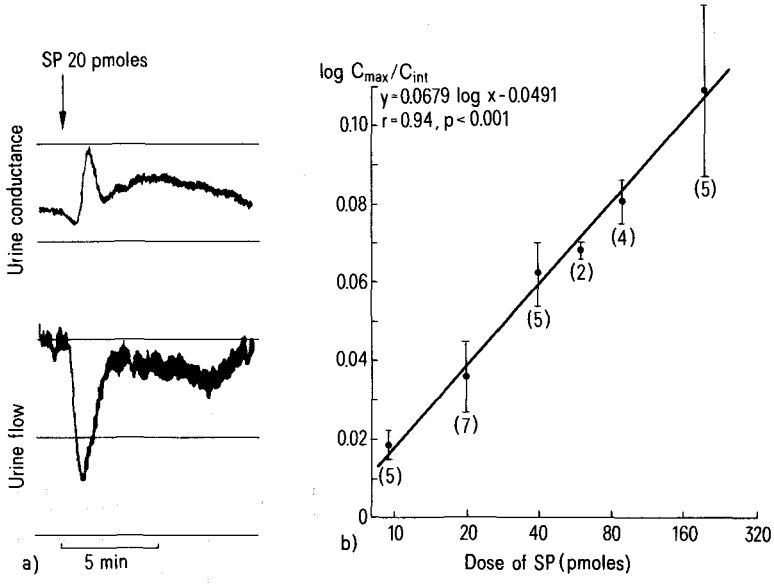


Fig. 2. *a* Effect of i.v. injection of SP on urine conductance and urine flow in an ethanolized rat. *b* Relationship between the log dose of SP and the log C_{max}/C_{int}, showing a linearity. The regression line is characterized by the formula $Y = 0.0679 \log X - 0.0491$ (X: dose of SP) and the index of precision ($\lambda = 0.17$).

the 2 acyl residues. Our results on the acylation-activity relationship are consistent with the study[25] which showed an increase in biological activity on the guinea-pig ileum, when the C-terminal SP fragments were substituted with the acyl group at N-terminal. Although the acylation was originally attempted to prevent degradation of SP fragments, in vivo half-lives of the nucleoside peptides have not been determined as yet.

The hypotensive activity of ARA-SP_{6-11} tended to be stronger than that of SP although the difference was not significant. The hypotensive activity of other analogs showed a potency of the same order as the antidiuretic activity.

In terms of time course, the maximal hypotensive effect occurred usually within 30 sec after SP, which preceded the maximal antidiuretic effect. It is likely that the hemodynamic effect may be partly responsible for the antidiuretic effect. However, to explain the discrepancy between the antidiuretic and hemodynamic potencies, the effects of SP and analogs on renal circulation[26] or glomerulo-tubular function[9] must be elucidated in detail.

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Adenylate cyclase of a human medullary thyroid carcinoma[1]

S. Matsukura, T. Kakita[2], M. Fukase and T. Fujita

Third Division, Department of Medicine, Kobe University School of Medicine, Ikuta-ku, Kobe 650 (Japan), 25 March 1980

Summary. The adenylate cyclase of a human medullary thyroid carcinoma was activated by TRH, glucagon, epinephrine, norepinephrine, phentolamine, serotonin and NaF, suggesting the presence of multiple hormone receptors including a β-adrenergic one in the tumor.

The adenylate cyclase-cyclic adenosine 3′, 5′-monophosphate (cyclic AMP) system is known to be involved in the secretory regulation of many hormones[3]. With respect to calcitonin (CT)[4,5] there is increasing evidence that the release of the hormone is modulated via the adenylate cyclase system in humans and animals. On the other hand, human medullary thyroid carcinoma[6,7] (MCT) secreting CT excessively has been found not to be completely autonomous in function but in part under the control of physiological stimuli. The present study was conducted to elucidate the adenylate cyclase response of MCT to various stimuli.

Materials and methods. A sporadic MCT which was later proven histologically was obtained from a 40-year-old woman who had hypercalcitonemia. The tumor was immediately placed in saline at 4 °C and homogenized in iced buffer solution composed of 62.2 mM Tris-HCl and 15.5 mM theophylline at pH 7.4. The whole homogenate was used in triplicate for the adenylate cyclase assay, which was carried out according to a modification[8] of the method described by Schorr et al.[9]. In brief, the adenylate cyclase activity was assayed by incubating the tissue homogenates in a buffered solution containing an ATP regenerating system and 8-^{14}C-ATP, with or without the addition of the agents, for 20 min at 37 °C, and expressed as cyclic AMP (pmoles/mg protein) produced during a 20-min incubation, based on the conversion of ^{14}C-ATP to ^{14}C cyclic AMP. TRH was supplied by Tanabe, Osaka, ACTH (Cortrosyn) by Daiichi, Tokyo, and tetra-gastrin by Eizai, Tokyo. Bovine TSH and prolactin were provided by NIAMDD, NIH. Glucagon was obtained from Novo, Denmark. Phentolamine was supplied by Ciba-Geigy, propranolol by I.C.I., and prostaglandin E_1 (PGE_1) and PGE_2 by Ono, Osaka. Dopamine, carbachol, and serotonin were purchased from Nakarai, Kyoto, norepinephrine from Fluka and epinephrine from Merck. 8-^{14}C-ATP was purchased from New England Nuclear. Statistical analysis was performed using Student's t-test.

Results. Data on the adenylate cyclase of an MCT are shown in the table. The cyclase was stimulated by TRH, glucagon, epinephrine, norepinephrine, phentolamine, serotonin, and NaF. Propranolol, a beta-adrenergic antagonist, blocked the stimulation by epinephrine.

Discussion. Little is known about the detailed mechanism of the adenylate cyclase in the parafollicular cells of the thyroid. Hunt et al.[10] recently reported that the adenylate cyclase of a MCT was stimulated only by glucagon and NaF while CT, isoproterenol, PGE_1 and gastrin had no effect. The present study demonstrates cyclase activation by epinephrine, norepinephrine and phentolamine, in addition to glucagon and NaF, and the blockade of epinephrine-induced activation by propranolol indicates the β-adrenergic control of CT release in the human MCT. Also in normal subjects, isoproterenol, phentolamine and, surprisingly, methoxamine (α-adrenergic agonist) in addition to Ca infusion have been shown to increase plasma CT whereas propranolol and EDTA infusion were found to decrease them[11,12]. Of further interest in the present study is the blockade of phentolamine-induced tumor cyclase activation by dopamine since l-dopa, a dopamine precursor, was found to depress CT release from human MCT[13]. Glucagon, on the other hand, is reported to stimulate CT release from the tumor in vivo[6,7] and in vitro[14], and also in normal subjects[5,15]. The activation of tumor cyclase by TRH and serotonin demonstrated in the present study is also noteworthy.

Adenylate cyclase of a medullary thyroid carcinoma. Whole tissue homogenates were incubated with or without the addition of hormones during 20 min at 37°C. Adenylate cyclase activity is expressed as pmoles of cyclic AMP produced per mg protein per 20 min

Addition	Concentration	Adenylate cyclase activity (cyclic AMP pmoles/mg protein/20 min)
Control		167± 8[a]
ACTH	10^{-5} M	168± 14
TSH	10^{-5} M	198± 11
Prolactin	10^{-5} M	167± 8
TRH	1.4×10^{-5} M	227± 4***
Tetra-gastrin	10^{-5} M	151± 5
Glucagon	10^{-4} M	296± 12***
PGE_1	2.8×10^{-5} M	244± 37
PGE_2	2.8×10^{-5} M	159± 28
Epinephrine	10^{-5} M	243± 12**
+ Propranolol	10^{-5} M + 10^{-5} M	124± 4
Norepinephrine	10^{-5} M	207± 6*
Propranolol	10^{-5} M	150± 8
Phentolamine	10^{-4} M	260± 30*
Dopamine	2.6×10^{-5} M	158± 23
+ Phentolamine	2.6×10^{-5} M + 10^{-4} M	190± 11
Serotonin	1.2×10^{-5} M	290± 59*
Carbachol	10^{-5} M	167± 11
NaF	10^{-2} M	314± 28***

[a] Mean ± SE of triplicate determinations; [b] p-values (stimulated vs control values): * $p<0.05$, ** $p<0.02$, *** $p<0.01$.