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NEURORECEPTOR MECHANISMS IN BRAIN

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Edited by

Shozo Kito
University of the Air
Chiba, Japan

Tomio Segawa
Hiroshima University School of Medicine
Hiroshima, Japan

and

Richard W. Olsen
University of California–Los Angeles School of Medicine
Los Angeles, California

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FOREWORD

The Third International Symposium on Neurotransmitter Receptors was held in Hiroshima at a time when the entire field of neurotransmitter receptors in the brain is progressing at an unprecedented pace. The symposium also marked my retirement as Professor and Chairman of the Third Department of Internal Medicine, Hiroshima University School of Medicine, and a new beginning as a Professor of the University of the Air.

The symposium was remarkably successful, and there were enthusiastic responses from scientists all over the world, proving that the meeting was timely. The selected papers contained in this volume constitute a state-of-the-art survey of the most advanced aspects of neurotransmitter receptor mechanisms in the brain.

I owe thanks for the great success of the symposium to Prof. Richard Olsen of UCLA, Prof. Tomio Segawa of Hiroshima University, Prof. Kinya Kuriyama of Kyoto Prefectural University of Medicine, and Prof. Masaya Tohyama of Osaka University. I express my sincere gratitude to many friends for making this publication possible. I especially thank Dr. Rie Miyoshi, whose devoted efforts as secretary-general were vital to the success of the symposium. Dr. Miyoshi is currently an instructor in the Department of Pharmacology at Tokyo Women's Medical College. I would also like to acknowledge the excellent secretarial work of Misses Ritsuko Sato and Yuko Wakita.

My appointment to the University of the Air affords me access to a well-equipped laboratory for basic biological research. I hope that my concentration on receptor studies over the next several years will make it possible for me to organize the Fourth International Symposium on Neurotransmitter Receptors, probably not at Hiroshima, but at the University of the Air, which is located close to the Tokyo metropolitan area.

Finally, this symposium was financially supported by the Japanese Ministry of Science, Culture, and Education.

Shozo Kito

PREFACE

The Third International Symposium on Neurotransmitter Receptors, held in Hiroshima, Fabruary 5-8, 1990, continues the tradition of the first two such symposia held in 1983 and 1987, also published by Plenum Press. However, the fame of these meetings has obviously been growing, as indicated by both the large number of abstracts received and the great number of countries represented by the attending neuroscientists.

The topics covered in this publication of the proceedings include structural and functional studies on some well-characterized receptor proteins, and characterization of receptor-coupling mechanisms, receptor interactions, and receptor regulation, with concentration on the central nervous system. Techniques described include now-classical molecular pharmacology through imaging of second messengers in cells and in intact brain to the most sophisticated use of recombinant DNA technology. Readers will no doubt find these representative chapters at the cutting edge of neuroscience.

Speaking on behalf of the participants at the Hiroshima symposium, I would like to thank Professor Kito sincerely and warmly for providing this excellent scientific program in the midst of an extremely friendly and enjoyable visit to Japan. I hope that there will be more such occasions.

Richard W. Olsen

CONTENTS

EFFECT OF NEUROPEPTIDES ON CLASSIC TYPES OF NEUROTRANSMISSION IN THE RAT CENTRAL NERVOUS SYSTEM

Shozo Kito and Rie Miyoshi

Third Department of Internal Medicine, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minamiku, Hiroshima 734 Japan

INTRODUCTION

Neuromodulator is a term for which there is no clear definition. For the past several years, the authors have been studing how neuropeptides modulate classic types of neurotransmission. We observed two examples of peptide action, that is, an effect of somatostatin on muscarinic acetylcholine receptors (mAChR) and that of cholecystokinin (CCK) on dopamine receptors in the rat brain, and obtained results suggesting that these two peptides modulated classical neurotransmitter receptors in a similar manner.

Both acetylcholine (Coyle et al., 1983; Perry et al., 1977) and somatostatin (Beal et al., 1986; Roberts et al., 1985) were definitely diminished in the brain of Alzheimer's disease. Morphologically, it has been reported that septo-hippocampal cholinergic neurons project to somatostatin-containing neurons in the hippocampus (Yamano and Luiten, 1989). In addition, it has been reported that somatostatin affects the turnover rate of acetylcholine in this brain area (Wood et al., 1981). Within the hippocampus, there are abundant M1 subtype mAChR (Vickroy et al., 1984). The authors investigated a modulatory effect of somatostatin on mAChR and an intracellular response after somatostatin receptor activation in the rat hippocampus from the viewpoint of pharmacology.

CCK receptors are observed richly in the striatum where dopaminergic nerve terminals ascending from the substantia nigra are observed with high density (Gaudreau et al., 1985). There has been strong evidence for dopamine and CCK interaction in the basal ganglia. For instance, CCK coexists with dopamine in the midbrain region (Hommer et al., 1986), and the peptide modulates dopamine release in the striatum (Hokfelt et al., 1985; Kovacs et al., 1981; Starr, 1982). Dopamine receptors have been classified into two major subtypes on the basis of their pharmacological and biochemical characteristics (Leff and Creese, 1983; Stoof and Kebabian, 1984). The functional significance of D2 receptors in the central nervous system has been well discussed from the viewpoints of pharmacotherapy of psychosis and movement disorders. Recently, availability of D1 selective ligands has made it possible to study physiological roles of D1 receptors in the central nervous system (Iorio et al., 1983). In the present paper, the effect of CCK on D1

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receptor binding and the intracellular transduction system of CCK receptors were examined in the rat striatum.

MATERIALS AND METHODS

Effect of Somatostatin on mAChR in the Hippocampus

Wistar strain male rats weighing 200-250 g were used in these experiments. After decapitation, the hippocampus was rapidly removed and P_2 fractions of the tissue were prepared. Both ^{3}H-QNB and ^{3}H-N-methyl-scopolamine (NMS) were used as mAChR antagonists. For observation of mAChR agonist binding sites, oxotremorine/^{3}H-NMS inhibition experiments were performed. As assay medium, Krebs-Henseleit solution containing bovine serum albumin, bacitracin and pepstatin was used. Non-specific binding was defined as the binding in the presence of 1 μM atropine. Aliquots of the tissue preparation were incubated at 30°C for 15 min. Membrane bound ^{3}H-ligands were trapped by the rapid vacuum filtration method.

The phosphatidylinositol (PI) turnover is one of the important second messenger systems involving an increase of intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) and activation of protein kinase C (Berridge and Irvine, 1984; Nishizuka, 1986). There has been strong evidence that mAChR activate this signal transduction system in various tissues (Brown et al., 1984; Janowsky et al., 1984). In this experiment, the synergistic effect of carbachol, a mAChR agonist, and somatostatin was investigated using hippocampal slices according to Berridge's method.

In addition, changes of $[Ca^{2+}]i$ in response to somatostatin were examined by fura-2 fluorometry on a single cell basis. Hippocampal neurons were obtained from 18-day rat embryos and dissociated cell cultures were made. Cultures were maintained for 12-14 days. After 3 days in vitro, cultures were treated with 10 μM cytosine arabinofuranoside for 24 hrs in order to suppress growth of fibroblasts and glial cells. The level of $[Ca^{2+}]i$ was measured in combination of fura-2, a fluorescence microscope, a video camera and photometrical devices as previously described (Kudo et al., 1986). Drugs used in this experiment were applied to cultured neurons by perfusion. During measurement, 1 μM tetrodotoxin was added to a buffer in order to block spontaneous Ca^{2+} flux. The amount of $[Ca^{2+}]i$ was quantitatively determined by a dual beam excitation method.

Effect of CCK on Dopamine D1 Receptors in the Striatum

For dopamine D1 receptor binding, the P_2 fraction of the striatum was used. ^{3}H-SCH23390 was used as a D1 selective antagonist. For observation of D1 antagonist binding sites, saturation experiments with ^{3}H-SCH23390 were performed. Non-specific binding was defined as binding in the presence of 10^{-5} M SCH23390. For D1 agonist binding sites, dopamine/^{3}H-SCH23390 inhibition experiments were done. As assay medium, 50 mM Tris HCl buffer, pH7.4 containing 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, bovine serum albumin and bacitracin was used. Aliquots of the tissue preparation were incubated at 22°C for 30 min.

To study intracellular responses of CCK receptors, changes of the PI turnover-Ca^{2+} signalling system in response to either CCK-8 or ceruletide, an octapeptide analog of CCK-8, were examined with use of the rat striatum.

Effect of Somatostatin on mAChR in the Hippocampus

Somatostatin had no effect on both ^{3}H-QNB and ^{3}H-NMS binding in the rat hippocampus (data not shown). However, as shown in Fig. 1, the peptide affected the affinity of agonist binding sites. An oxotremorine/^{3}H-NMS inhibition curve exhibited heterogeneous characteristics with a Hill coefficient much less than 1. The binding data were fitted best by a two-site model, characterized by two dissociation constants: a K_H value of 6.6×10^{-9} M and a K_L value of 2.6×10^{-6} M. Percentages of high and low affinity binding sites to the total binding capacity were 36.3 % and 63.7 %, respectively. The inhibition curve after adding 1 μM [D-trp^{8}]somatostatin in the incubation medium exhibited a Hill slope factor close to 1, and the curve fitted best to a binding model consisting of a single homogeneous binding site whose Ki value was consistent with the K_L value.

As previously reported by various groups (Brown et al., 1984; Janowsky et al., 1984), carbachol caused an enhancement of ^{3}H-inositol-1-phosphate (IP_1) accumulation. Somatostatin significantly augmented the effect of carbachol ($p<0.01$), while somatostatin itself had no effect on the basal accumulation of ^{3}H-IP_1 (Fig. 2).

As the next step, to elucidate the mechanism of the above-mentioned effects of somatostatin on mAChR, changes of $[Ca^{2+}]i$ in response to somatostatin were examined in cultured hippocampal neurons. Fig. 3 shows the response to either [D-trp^{8}]somatostatin or SMS201-995 at the concentration of 10^{-5} M in the same cultured hippocampal neuron. The basal level of $[Ca^{2+}]i$ in neurons was about 100 nM. By perfusing with [D-trp^{8}]somatostatin for 30 sec., a monophasic increase of $[Ca^{2+}]i$ was observed. The cell population which responded to [D-trp^{8}]somatostatin stimulation was 26.5 % of the total neurons. SMS201-995, a very potent octapeptide analog of somatostatin, had about 2 times more potency on elevation of $[Ca^{2+}]i$. This effect of somatostatin was found to be dose-dependent when concentrations ranging from 10^{-8} to 10^{-4} M were used. As next experiments, the Ca^{2+} source for the effect of somatostatin was determined. Fig. 4 shows changes of $[Ca^{2+}]i$ in response to [D-trp^{8}]somatostatin in cases of perfusing with either normal buffer or Ca^{2+}-depleted medium in the same neuron. The effect of somatostatin was completely blocked in Ca^{2+}-depleted medium. The

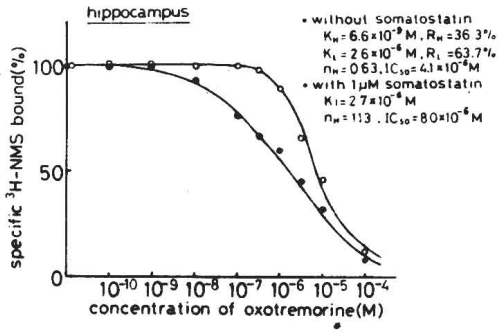

Fig. 1 Oxotremorine binding to P_2 fraction of the rat hippocampus measured by competition with ^{3}H-NMS in the presence and absence of 1 μM [D-trp^{8}]somatostatin. n_H: Hill coefficient. The concentration of ^{3}H-NMS was 0.2 nM. These values were means in 3 repeated experiments.

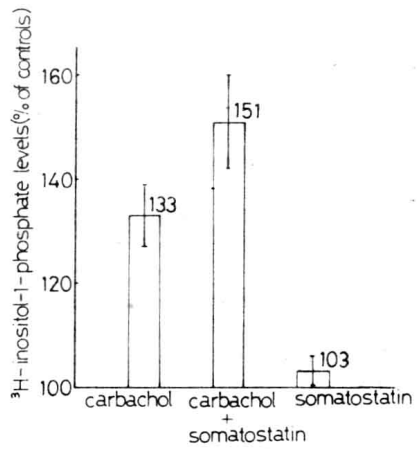

Fig. 2 Effect of [D-trp^8]somatostatin on carbachol-stimulated inositol phospholipid hydrolysis in rat hippocampal slices. The concentrations of carbachol and somatostatin were 1 mM and 50 μM, respectively. Somatostatin augmented carbachol-induced accumulation of ^{3}H-IP$_1$ in a dose-dependent manner (data not shown). Values represent % of those in experiments in which 10 mM LiCl alone was added to the incubation medium. $p<0.05$, vs the data with 1 mM carbachol. n=3.

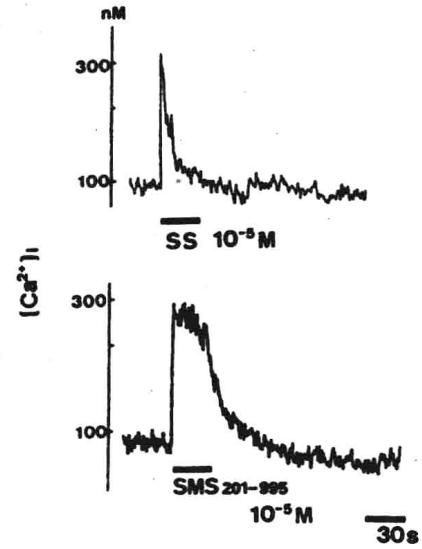

Fig. 3 Effect of 10^{-5} M [D-trp^{8}]somatostatin (SS) or SMS201-995 on $[Ca^{2+}]i$ in the same cultured rat hippocampal neuron. Drugs were applied to the neuron by perfusion for 30 sec.

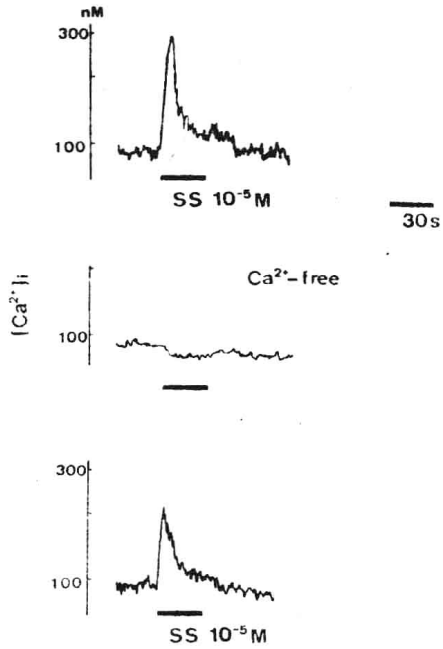

Fig. 4 Determination of the source of Ca^{2+} for [D-trp^{8}]somatostatin (SS)-induced elevation of $[Ca^{2+}]i$ in cultured rat hippocampal neurons. The upper plate shows a result of an experiment perfusing with normal buffer and the middle Ca^{2+}-depleted medium. In the latter case, the neuron was perfused for 1 min with Ca^{2+}-depleted medium in which $CaCl_2$ was removed and 0.1 mM EGTA was added, prior to SS stimulation. The lower plate shows recovery of the response by re-perfusing with normal buffer.

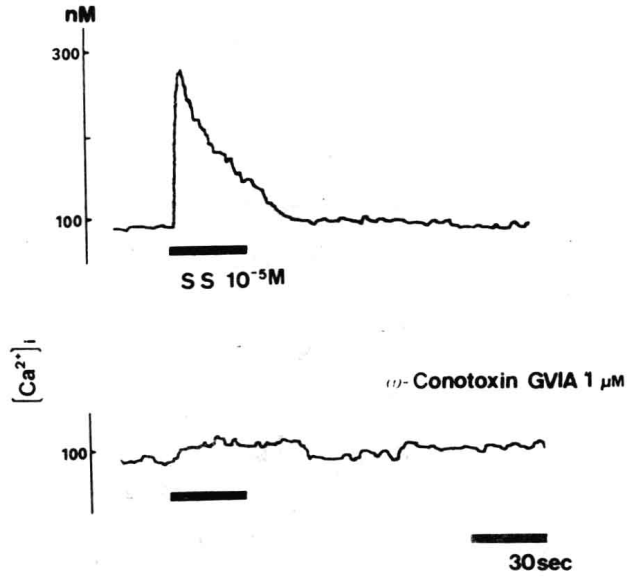

Fig. 5 Effect of ω-conotoxin GVIA on [D-trp^{8}]somatostatin (SS)-induced increase of $[Ca^{2+}]i$ in cultured rat hippocampal neurons. The upper plate shows a result of an experiment perfusing with normal buffer and the lower that of pretreatment with 1 μM ω-conotoxin GVIA for 1 min. A set of experiments were performed using the same neuron.

binding sites without changing dissociation constants, independently. In the presence of NaCl, CCK-8 caused further conversion to a low affinity state. However, when both GTP and CCK-8 were added, CCK-8 did not convert anymore.

Effect of CCK on Dopamine D1 Receptors in the Striatum

Scatchard analysis revealed that ^{3}H-SCH23390 had a single high affinity binding site whose Kd and Bmax values were 1.57 nM and 907.6 fmol/mg protein, respectively. CCK-8 at the concentration of 1 μM had no effect on ^{3}H-SCH23390 binding (data not shown).

Table 1 shows results obtained from dopamine/^{3}H-SCH23390 inhibition experiments in the rat striatum. Curves without and with CCK-8 exhibited heterogeneous characteristics with Hill coefficients much less than 1. In the absence of CCK-8, the binding data were fitted best by a two-site model, characterized by two dissociation constants: a K_H value of 7.76×10^{-7} M and a K_L value of 2.45×10^{-5} M. Percentages of high (R_H) and low (R_L) affinity binding sites to the total binding capacity were 74.6 and 25.4 %, respectively. After adding 1 μM CCK-8, R_H decreased to 51.3 % and R_L increased to 48.7 %, with their affinity constants unchanged. Guanine nucleotides and sodium ion have been known to convert agonist high affinity binding sites into low affinity ones in striatal dopamine receptors (Grigoriadis and Seeman, 1985). The mechanism of the effect of guanine nucleotides on agonist binding has been well investigated and accepted to be the result of the dissociation of a GTP binding protein from a receptor protein. The mechanism of sodium ion is not yet clear, but it is suggested that sodium ion acts like an allosteric inhibitor of dopamine receptors. In our experiments, both 50 μM GTP and 120 mM NaCl caused a reduction of high affinity

Table 1. Effect of CCK-8, NaCl and GTP on dopamine binding to the rat striatum measured by competition with ^{3}H-SCH23390

	n_H	$-\log[K_H]$	$-\log[K_L]$	R_H (%)	R_L (%)
	0.64 ± 0.10	6.11 ± 0.53	4.61 ± 0.17	74.60 ± 1.44	25.40 ± 1.44
CCK-8	0.75 ± 0.06	7.02 ± 0.09	5.67 ± 0.14	51.27 ± 5.83	48.73 ± 5.83
NaCl	0.67 ± 0.04	6.17 ± 0.01	4.73 ± 0.10	55.61 ± 0.81	44.39 ± 0.81
NaCl + CCK-8	0.73 ± 0.02	6.91 ± 0.26	5.05 ± 0.04	27.61 ± 4.81	72.39 ± 4.81
GTP	0.71 ± 0.20	6.99 ± 1.47	4.93 ± 0.49	48.13 ± 7.53	51.87 ± 7.53
GTP + CCK-8	0.70 ± 0.04	6.97 ± 0.43	5.41 ± 0.14	45.94 ± 9.29	54.06 ± 9.29

Synergistic effects of CCK-8 and NaCl or GTP on dopamine/^{3}H-SCH23390 inhibition experiments were studied in rat striatal homogenates. These drugs were added to the incubation medium. These values were means of 3 repeated experiments.

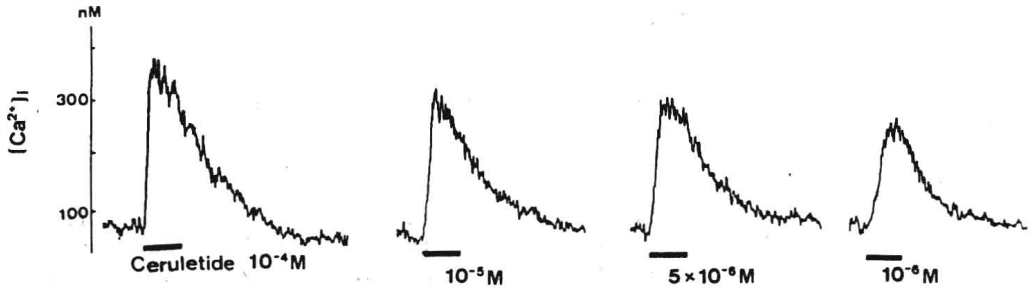

Fig. 6 Effect of various concentrations of ceruletide on $[Ca^{2+}]i$ in the same cultured rat striatal neuron. Ceruletide was applied to the neuron by perfusion for 30 sec.

blockage was recovered by re-perfusing with the normal buffer. After 1 min pretreatment with 1 μM ω-conotoxin GVIA, a neurotoxic peptide from the venom of a marine snail, the effect of somatostatin was completely inhibited (Fig. 5). Nifedipine, one of the dihydropyridine Ca^{2+} channel blockers, had no effect on the $[Ca^{2+}]i$ rise induced by somatostatin.

Using the same experimental process as in the case of somatostatin, the authors investigated the effect of CCK on the PI turnover-Ca^{2+} signalling system in the rat striatum. Neither CCK-8 nor ceruletide changed basal PI turnover. However, ceruletide caused a monophasic increase of $[Ca^{2+}]i$ in a dose-dependent manner as shown in Fig. 6. The response to ceruletide was completely blocked by perfusing with Ca^{2+}-depleted medium or pretreatment with ω-conotoxin GVIA.

DISCUSSION

Since the effect of somatostatin on mAChR in the rat hippocampus was limited to agonist binding, it seems that somatostatin dose not bind mAChR directly, but the peptide affects the functional state of receptors through some intracellular responses.

Somatostatin has been considered to be an inhibitory neuropeptide throughout the endocrine and nervous systems. As for the intracellular response, somatostatin inhibits adenylate cyclase activity and Ca^{2+}

mobilization in the pituitary, pancreas and so on (Catalan et al., 1979; Chneiweiss et al., 1987; Diez and Tamargo, 1987; Luini et al., 1986). In our experiments, in the presence of somatostatin, the muscarinic function seems to be activated, that is, a reduction of the affinity of receptors and augmentation of PI turnover elicited by a mAChR agonist in the hippocampus occur. In previous reports, an excitatory effect of somatostatin has been described in the hippocampus and cerebral cortex from viewpoints of electrophysiology (Dodd and Kelly, 1978; Ioffe et al., 1978; Olpe et al., 1980). In this paper, as a novel excitatory response of somatostatin, the authors obtained results that the peptide increased $[Ca^{2+}]i$, probably via N-type Ca^{2+} channels. It is considered that an increase of $[Ca^{2+}]i$ induced by somatostatin is a key event for modulating the muscarinic function.

Galanin is a neuropeptide which is considered to have some relation to the septo-hippocampal cholinergic system (Dutar et al., 1989; Melander et al., 1985; Senut et al., 1989). Consolo et al. reported that galanin reduced PI turnover elicited by carbachol probably because of its lowering action of $[Ca^{2+}]i$ in the rat hippocampus (Consolo et al., 1989). The authors confirmed that galanin decreased $[Ca^{2+}]i$ stimulated by high K^{+} (unpublished data). A possible mechanism on modulation of hippocampal mAChR by neuropeptides is schematically shown in Fig. 7.

Our indirect agonist binding studies revealed that CCK-8 converted a part of D1 agonist high affinity binding sites into low affinity ones in the rat striatum. It was noticed that the effect of CCK-8 was similar to that of GTP rather than that of NaCl. Therefore, it is considered that CCK-8 modulates D1 agonist binding sites in a GTP-like manner. It is noteworthy that somatostatin and CCK exert the same kind of effect on classical neurotransmission, that is, these two peptides reduce the affinity of agonist binding for muscarinic and D1 receptors,

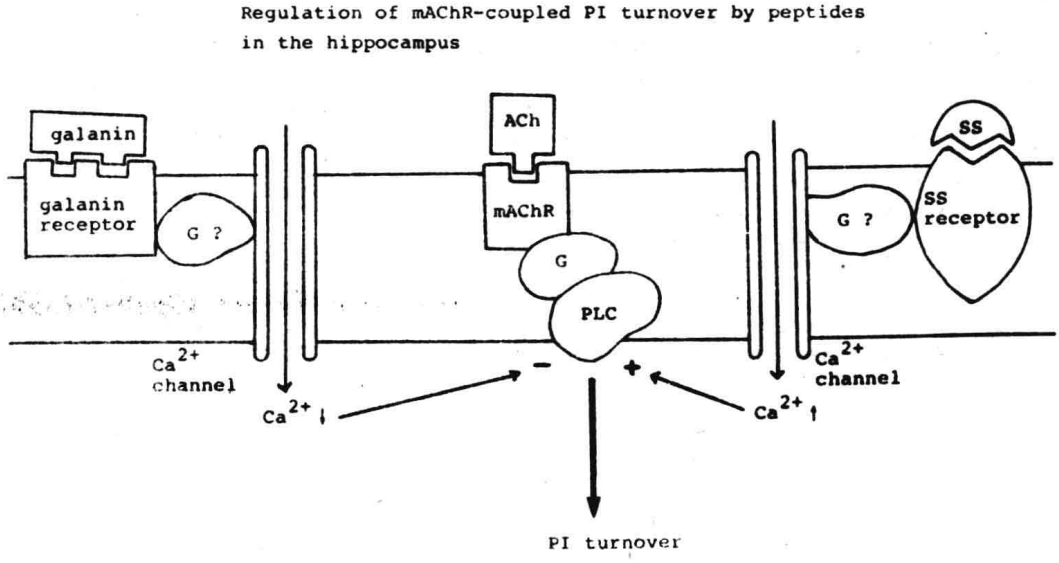

Fig. 7 A possible scheme for regulation of mAChR-induced stimulation of PI turnover by neuropeptides. It seems that the hippocampal mAChR function is regulated by at least two neuropeptides, that is, somatostatin and galanin, in an opposite manner.