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SHORT COMMUNICATIONS

Substance P inhibits catecholamine biosynthesis stimulated by carbamylcholine in cultured adrenal chromaffin cells

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Abstract—The effect of substance P on catecholamine biosynthesis was examined using cultured bovine adrenal chromaffin cells as a model for the sympathoadrenergic system. Substance P markedly inhibited the formation of [^{14}C]catecholamines from L-[^{14}C]tyrosine stimulated by cholinergic agonist, but caused no significant effect on the biosynthesis stimulated by depolarizing agent. In addition, this inhibitory action was completely prevented by the addition of substance P antagonists. Under the conditions in which the inhibition of catecholamine biosynthesis was observed, substance P also inhibited the influx of extracellular $^{45}Ca^{2+}$ into these cells, and this inhibitory action on Ca^{2+} influx was almost identical to that on the biosynthesis. These results provide evidence for a possible role of substance P as a putative neuromodulator in the sympathoadrenergic system.

Catecholamine secretion evoked by stimulation of acetylcholine receptors from adrenal medulla cells has been shown recently to be inhibited by substance P [1–3], which is well known to coexist with acetylcholine in the splanchnic nerve [4–6]. In contrast, substance P has been reported to enhance the secretory responses to prolonged or repeated stimulation of the cell as a result of preventing the desensitization of acetylcholine receptors [7–9]. This peptide is therefore thought to play a physiologically important role as one of the putative neuromodulators in the sympathoadrenergic system. On the other hand, it has been well established that the stimulation of catecholamine secretion is accompanied by the stimulation of catecholamine biosynthesis, which is also known to require the presence of extracellular Ca^{2+} [10]. It therefore seems reasonable to presume that the agents modulating catecholamine secretion may have some influence on catecholamine biosynthesis in various sympathoadrenergic tissues.

In the present study, we examined the effect of substance P on catecholamine biosynthesis in primary cultured adrenal chromaffin cells, and found that this peptide could inhibit the formation of [^{14}C]-catecholamines from L-[^{14}C]tyrosine as a result of the inhibition of extracellular Ca^{2+} influx into the cells under the conditions in which its inhibitory action on catecholamine secretion was observed.

Materials and Methods

Chromaffin cells were prepared enzymatically from fresh bovine adrenal medulla and cultured for 3–4 days as described previously [11]. Cells were washed with balanced salt solution [135 mM NaCl, 5.6 mM KCl, 1.2 mM $MgSO_4$, 2.2 mM $CaCl_2$, 10 mM glucose and 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid/NaOH, pH 7.4], and then stimulated by various secretagogues at 37° for 30 min in 0.5 mL of balanced salt solution containing 20 μM L-[^{14}C]tyrosine (0.25 μCi/well) with or without substance P. After discarding the incubation medium by aspiration, the cells were washed twice with 1 mL of ice-cold balanced salt solution and then lysed by adding 0.5 mL of 0.4 M perchloric acid. The cell lysate was centrifuged at 9000 *g* for 5 min and the radioactivity in a 50 μL aliquot of the supernatant fraction was then counted using a liquid scintillation spectrometer to determine the uptake of L-[^{14}C]tyrosine into the cells. Radioactive catecholamines in the rest of the acid extract were isolated onto aluminum hydroxide gel, as reported previously [12], and the radioactivity eluted from the gel was then determined by liquid scintillation spectrometry.

To examine the effect of substance P on Ca^{2+} influx, the cells were washed and stimulated by various secretagogues in 0.25 mL of balanced salt solution containing $^{45}CaCl_2$ (4 μCi/mL) with or without this peptide. At the end of the incubation period, the medium was discarded and the cells were washed four times with 1 mL of ice-cold Ca^{2+}-free balanced salt solution and then solubilized with 0.5 mL of 1% Triton X-100. Radioactivity in the cell lysate was determined by liquid scintillation spectrometry.

L-[U-^{14}C]tyrosine and $^{45}CaCl_2$ were purchased from the New England Nuclear Corp. (Boston, MA, U.S.A.). Substance P and its antagonists were purchased from the Peptide Institute Inc. (Osaka, Japan). Carbamylcholine was obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Other chemicals used were of commercially available reagent grade.

Results and Discussion

The effect of substance P on the production of [^{14}C]-

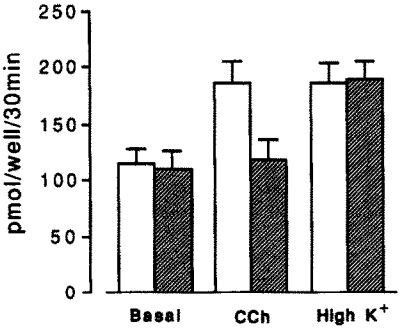

Fig. 1. Effect of substance P on the production of [^{14}C]-catecholamines in cultured adrenal chromaffin cells. Cells were incubated with 300 μM carbamylcholine or high K^+ (56 mM KCl) in the mixture containing [^{14}C]tyrosine with (hatched bar) or without (open bar) 10^{-5} M substance P, and the amounts of [^{14}C]catecholamines formed during the incubation period were then determined as described in the text. Values are the means ± SE of six experiments.

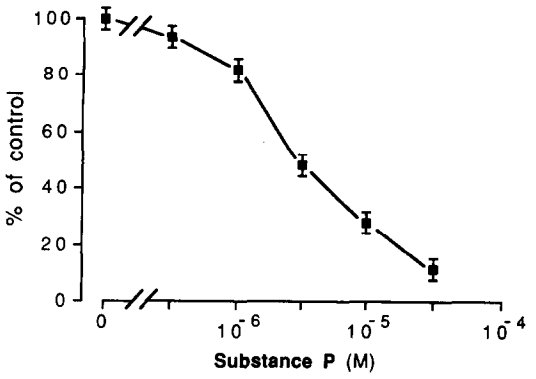

Fig. 2. Effect of substance P on the production of [^{14}C]-catecholamines in cultured adrenal chromaffin cells as a function of the concentration. Cells were stimulated by 300 μM carbamylcholine in the mixture containing [^{14}C]-tyrosine with various concentrations of substance P, and the amounts of [^{14}C]catecholamines were then determined as described in the text. Values are the means ± SE of six experiments.

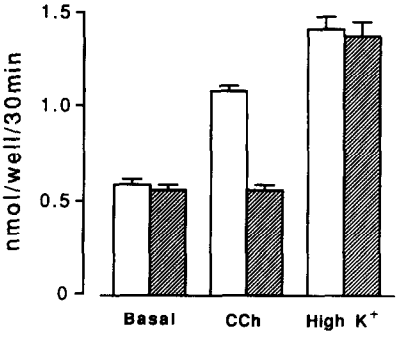

Fig. 3. Effect of substance P on $^{45}Ca^{2+}$ uptake into cultured adrenal chromaffin cells. Cells were stimulated by 300 μM carbamylcholine or high K^+ (56 mM KCl) in the mixture containing $^{45}CaCl_2$ with (hatched bar) or without (open bar) 10^{-5} M substance P, and the amount of $^{45}Ca^{2+}$ taken up into the cells was then determined as described in the text. Values are the means ± SE of six experiments.

catecholamines from L-[^{14}C]tyrosine in cultured bovine adrenal chromaffin cells was examined to obtain further information on a possible role of this peptide as a putative modulator for the sympathoadrenergic function. As shown in Fig. 1, the carbamylcholine-stimulated production of [^{14}C]catecholamines was almost completely inhibited by substance P, whereas neither the basal nor the high K^+-stimulated level of [^{14}C]catecholamine production was significantly affected by this peptide. The inhibitory action of substance P on catecholamine biosynthesis stimulated by carbamylcholine was observed in a manner dependent on its concentration (Fig. 2). On the other hand, this peptide failed to cause any significant effect on the uptake of L-[^{14}C]tyrosine into the cells and caused no direct action on the activity of tyrosine hydroxylase prepared from bovine adrenal medulla (data not shown). Substance P was therefore considered to inhibit the production of [^{14}C]-catecholamines as a consequence of inhibiting the stimulatory action of carbamylcholine on the cell surface rather than the intracellular processes of catecholamine biosynthesis.

Substance P has been shown previously to inhibit catecholamine secretion evoked by cholinergic agonists, but not by depolarizing agents [1–3]. We examined again the effect of this peptide on catecholamine secretion under these experimental conditions to examine its action on the biosynthesis. Substance P also inhibited catecholamine secretion evoked by carbamylcholine, and this inhibitory action on the secretion was identical with that on the biosynthesis shown in Figs 1 and 2 (data not shown). It therefore seemed that substance P might act on the common process which is presumably involved in the stimulatory actions of cholinergic agonists on both the biosynthesis and the secretion of catecholamines in adrenal chromaffin cells.

As catecholamine biosynthesis is known to be tightly coupled to catecholamine secretion and these processes require the presence of Ca^{2+} in the extracellular space [10], the effect of substance P on the influx of extracellular Ca^{2+} into the cells was examined. The $^{45}Ca^{2+}$ uptake stimulated by carbamylcholine, but not by high K^+, was completely inhibited by substance P (Fig. 3) and this inhibitory action was similar to that on catecholamine production, shown in Fig. 1. Furthermore, the inhibitory potency of substance P on biosynthesis as well as secretion was not altered by varying the concentration of carbamylcholine (data not shown). The inhibitory action of substance P on catecholamine biosynthesis stimulated by carbamylcholine was therefore considered to be due to its action on the acetylcholine receptor-linked cation channels rather than its competitive inhibition of acetylcholine.

To confirm that the inhibition of catecholamine biosynthesis observed here can be attributed to the action of substance P itself, the inhibitory action of this peptide on [^{14}C]catecholamine production was examined again in the presence of substance P antagonists such as [D-Pro^2,D-Trp^{7,9}] substance P and [D-Arg^1,D-Trp^{7,9},Leu^{11}] substance P. The inhibitory action of substance P on catecholamine biosynthesis was completely abolished by the addition of these antagonists at 3×10^{-5} M (data not shown). It therefore seemed likely that substance P might cause the inhibition of catecholamine biosynthesis through direct action on the substance P receptors.

The present study shows that substance P caused the inhibition of catecholamine biosynthesis evoked by stimulation of the acetylcholine receptors as a consequence of inhibiting the influx of Ca^{2+} into the cells, and this inhibitory action is considered to be due to its action on Ca^{2+} transport which might be activated by stimulation of the acetylcholine receptors in adrenal chromaffin cells. In view of previous findings that substance P can modulate the secretory responses of adrenal medulla cells to stimulation of the acetylcholine receptors, the results presented here suggest that substance P may play a role in modulating the function of the sympathoadrenergic system.

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