RAPID COMMUNICATION

Stimulation of Immunoreactive Somatostatin Release from Hypothalamic Synaptosomes by High (K^+) and Dopamine

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Synopsis

Effects of high (K^+) and dopamine on the release of immunoreactive somatostatin from isolated hypothalamic synaptosomes were studied in rats. High (K^+) (60 mM) and dopamine (10^{-6} M) in the incubation media stimulated the release of immunoreactive somatostatin and the former effect was completely abolished by the removal of Ca^{++} from the media. These suggest that hypothalamic somatostatinergetic synaptosomes preserved at least one of the important basic properties of secretory cells. Although it is of interest to note that dopamine stimulated the release of somatostatin. Its physiological significance awaits further studies.

The pituitary stalk and the median eminence (SME) region contains the largest amount of somatostatin in the central nervous system (Brownstein *et al.*, 1975). Utilizing immunohistochemical technique, somatostatin present in SME region was observed to be contained largely in synaptosomes (Hököfelt *et al.*, 1975). This was proven recently by the direct measurement of the subcellular distribution of somatostatin in the rat SME using radioimmunoassays (Epelbaum *et al.*, 1977).

Short-term incubations of isolated synaptosomes have been used to study the effects of putative neurotransmitters on the release of other hypothalamic releasing hormones (De Bellerocche *et al.*, 1973; Bennett *et al.*, 1975). To our knowledge, there appeared no such reports to observe the release of immunoreactive somatostatin from isolated hypothalamic synaptosomes.

The present experiments deal with the effects of high (K^+) and Ca^{++} on the

release of somatostatin from isolated hypothalamic synaptosomes. In addition, the effect of dopamine on the release of somatostatin from isolated hypothalamic synaptosomes was examined.

Materials and Methods

Male Wistar rats weighing 200 g were used. They were maintained in an air-conditioned animal house on a lighting schedule of 12 hr light and 12 hr darkness and fed *ad libitum*. Rats were sacrificed by decapitation and brain was removed immediately. Fragments of the pituitary stalk and the hypothalamic tissue (measuring 4 mm in diameter and 3 mm in thickness with the pituitary stalk remnant as a central reference point) were excised. They were placed in an ice cold 0.3 M sucrose (10% W/V). Synaptosomes were prepared by homogenization and density gradient centrifugation according to the method of Gray and Whittaker (1962) as modified by Hájós (1975). The preparation from the hypothalamus was examined by electron microscopy and found to be rich in synaptosomes.

Synaptosomes thus obtained were suspended in Krebs-Ringer bicarbonate medium containing 10 mM glucose (KRBG) at a concentration of 1.2 hypothalamic equivalent per ml. When high K^+ concen-

tration was used, sodium chloride was replaced with an equimolar amount of potassium chloride. To prepare calcium-free media, solutions were made by osmotically equivalent replacement of CaCl_2 with NaCl . Samples (1 ml) of synaptosomes suspensions were incubated for 30 min at 37°C in a metabolic shaker under the atmosphere of 95% O_2 and 5% CO_2 . Dopamine HCl was dissolved in 5% glucose solution and was added to synaptosomes suspensions in a volume of $10\ \mu\text{l}$ at the beginning of the incubation. After the incubation, synaptosomes were removed by centrifugation at 20,000 rpm for 5 min and the supernatants were stored at -80°C until they were assayed for somatostatin contents.

Somatostatin contents in the incubation media were measured by a specific radioimmunoassay method developed by Arimura *et al.* (1975). The antibody was generously supplied by Dr. Arimura. N-tyr-somatostatin and synthetic somatostatin used in the assay were kindly supplied by Dr. Coy. The sensitivity of the assay was 3.9 pg, with an intraassay variation of coefficient of 6.5%. Each sample was assayed for somatostatin in triplicates. The values were pooled by the treatment groups and means and standard errors were calculated. Statistical analysis of the data was performed by Duncan's new multiple range test (Steel and Torrie, 1960) or Student *t* test.

Fig. 1. Amounts of somatostatin determined, as a function of the dilution of the incubation medium where hypothalamic synaptosomes have been incubated in the radioimmunoassay for somatostatin.

Results

As shown in Fig. 1, measured concentrations of immunoreactive somatostatin in the incubation media fell linearly with dilutions, suggesting that the material assayed was indistinguishable immunologically from synthetic somatostatin. Though data were not shown, the presence of 10^{-6}M dopamine or 60 mM K^+ within assayed volumes did not interfere with the radioimmunoassay at all.

Fig. 2 shows the result of the experiment in which (K^+) in the medium was increased by 10-folds. The amount of immunoreactive somatostatin released from isolated

Fig. 2. Effects of high- (K^+) KRBG and Ca^{++} on the release of immunoreactive somatostatin (SRIF) from hypothalamic synaptosomes. In this and the subsequent figure, the height of the column indicates the mean and the vertical bar above indicates one standard error of the mean of *n* observations.

NS: not significant

hypothalamic synaptosomes increased significantly in the incubation media containing 60 mM K^+ . Removal of Ca^{++} from high- (K^+) KRBG prevented completely its stimulatory effect.

Fig. 3 shows the effect of dopamine on the release of immunoreactive somatostatin from isolated hypothalamic synaptosomes. When 10^{-6} M dopamine was present in the incubation media, the amount of somatostatin released from hypothalamic synaptosomes increased significantly.

Discussion

Depolarization of the plasma membrane and the subsequent increase of Ca^{++} influx into the cell is believed to be an important event in the release of secretory products from cells (Douglas, 1968). Our present results showed that high K^+ concentration in the media increased the release of im-

munoreactive somatostatin from hypothalamic synaptosomes and its effect was completely abolished by the removal of Ca^{++} from the media. This means that synaptosomes used in the experiment preserved at least one of the important basic properties of secretory cells. Further, it infers that the preparation used in the experiment can be used to test the effects of various putative neurotransmitters on the release of somatostatin.

In rats, the administration of dopaminergic agents exert the inhibitory action on the release of growth hormone (GH) (Collu *et al.*, 1972; Kato *et al.*, 1974), though contradicting data were reported by Smythe *et al.* (1975). At any rate, dopaminergic agents appear to exert their effects acting through the hypothalamus (Collu *et al.*, 1972; Kato *et al.*, 1974). However, it is largely unknown how somatostatin is involved in the mechanism whereby dopamine modulates the secretion of GH in rats. Thus, it is of interest to note that 10^{-6} M dopamine stimulated the release of somatostatin from hypothalamic synaptosomes. The result may give an insight into the mechanism whereby dopamine leads to the inhibition of GH secretion in rats. However, this interpretation has some reservations. In our studies, the dose-response relationship between dopamine added to the media and the amounts of somatostatin released was not performed. Also, whether or not the antidopaminergic agents block the dopamine-mediated somatostatin release was not tested. Dopamine in the salt solution is subject to the destruction (Takahara *et al.*, 1974). When 10^{-9} M dopamine was tested, the release of somatostatin was not observed consistently. Therefore, 10^{-6} M dopamine was chosen considering that the significant portion of which might be destroyed during the incubation period. KRBG was chosen as the media, since the modification of its ionic composition was achieved easily.

Fig. 3. Effect of dopamine on the release of immunoreactive somatostatin (SRIF) from hypothalamic synaptosomes.

However, when catecholamines such as dopamine were added to KRBG solution, it was realized that measures should be taken to prevent their oxidation. Therefore, further careful studies are necessary to decide whether or not the stimulation of somatostatin from hypothalamic synaptosomes by dopamine has physiological meanings.

As reported recently, (Griffiths *et al.*, 1977), synaptosomal fraction contains peptidasic activity which degrades somatostatin quickly. The small extent of stimulation of somatostatin release by high-(K⁺) media and dopamine observed in the present experiment could be due to such a peptidasic activity contained in the media.

In conclusion, our present results suggest that somatostatinergic synaptosomes isolated from SME preserved one of the important basic properties of the secretory cells and could be used to study the effects of various putative neurotransmitters on the release of somatostatin. Although dopamine 10⁻⁶ M stimulated the release of somatostatin from hypothalamic synaptosomes, the physiological significance of this finding should await further studies.

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