High K^+-induced release of somatostatin from the cortical preparation of rat brain[1]

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Summary. Release of endogenous somatostatin (SRIF) from the rat cerebral cortical slices incubated in Krebs-bicarbonate buffer was increased from the basal rate of 3.4±0.6% of the total SRIF content in 15 min at $[K^+]_o = 5.6$ mM, to 13.1±1.6% upon raising the $[K^+]_o$ to 56.6 mM. The high-K^+ evoked SRIF release was absent when Ca^{++} in the medium was replaced by Mn^{++}. The isolated synaptosomes from rat cerebral cortex contain 13.2±3.1 ng SRIF/mg protein compared to 0.33±0.01 ng/mg protein in the cortical tissue as a whole, suggesting that nerve terminals are the main source of the peptide released upon membrane depolarization.

Somatostatin (SRIF), a tetradecapeptide, was originally isolated from sheep and porcine hypothalami[3] and the function of this peptide found to be associated with the control of secretion of GH and TSH in pituitary gland[4] (hence the name growth hormone release-inhibiting hormone). However, subsequent studies have shown that the hormone peptide is present in significant quantities in various extrahypothalamic regions of the brain as well as in certain extra-neuronal locations in mammals[5–7].

In the brain the highest concentration of somatostatin is in the hypothalamus (1.11 ng/mg wet wt). In the cerebral cortex the concentration of somatostatin is relatively low (0.03 ng/mg wet wt). Nevertheless, due to the large size of the cerebral cortex the total amount of somatostatin in this structure (30.0 ng/region) is very similar to that in the hypothalamus (36.3 ng/region)[8]. Of particular interest are the histochemical findings of neurons in the cerebral cortex which contain immunoreactive somatostatin. Moreover, studies using immunohistochemical methods[10] and radioimmunoassay with synaptosomes[11] have shown that in the hypothalamus SRIF is highly concentrated in the nerve endings. These and similar observations have led to speculation that SRIF is a neurotransmitter and/or neuromodulator in the CNS[12]. Nevertheless, critical evidence for such a role, consistent with Dale's postulate[13], concerns the release of the putative transmitter from nerve-endings upon membrane depolarization. Calcium dependent release of SRIF in response to stimulation has been demonstrated in in vitro experiments in the neurohypophysis[14], in the cultured dorsal root neurons[15], in the hypothalamus[16,17] and in the amygdala[17]. Of particular interest is the response of the neocortex which, like the hypothalamus, is a major source of SRIF in the CNS. The present studies were designed to investigate the release of somatostatin from rat neocortical tissue in vitro and its calcium-dependence. The results obtained support the hypothesis of peptidergic neurotransmission and/or neuromodulation within the CNS, specifically in the neocortex.

Materials and methods. Male Spraque-Dawley rats weighing 200–250 g were decapitated; the whole cortex was dissected out and slices or strips approximately 1 mm in thickness were prepared. After allowing the brain preparations to stabilize for 15–30 min in the normal Krebs-bicarbonate buffer saturated with oxygen, pH 7.4, 37 °C, the incubation was started; the incubation medium was collected and replaced with fresh solution every 15 min (details of the incubation media are described in the legends). During the entire incubation the media were continuously gassed with 95% O_2-5% CO_2. The samples of incubation medium as well as the acetic acid extracts of the post-incubation brain tissues were boiled immediately. SRIF concentration was determined by a radioimmunoassay method similar to that described previously[18]. The method had a sensitivity of 10 pg SRIF/tube. The mean inter - and intraassay variation are 6.1% and 4%, respectively. The anti-SRIF antiserum used in the present study did not crossreact with other commonly encountered hormones or the biogenic amines. Protein concentration was determined by the method of Lowry et al.[19]. Results were analyzed by the analysis of variance and Duncan's multiple range test.

Results. The rates of SRIF release from the rat brain cortical slices during 15 min intervals of incubation of Krebs medium or a modified solution, containing 5.6 mM and 56.6 mM KCl, respectively, are shown in figure 1. In these experiments the brain preparations responded to the increase in K^+ concentration in the medium with a 2-fold increase in the release of SRIF. Moreover, given a 30 min recovery incubation in normal Krebs medium subsequent to a high-K^+ stimulation, the responsiveness of the brain tissue was restored. Although the magnitude of responses decreased with time, up to 3 serial high K^+-induced releases of SRIF could be produced within 2 h.

In the case of well known neurotransmitters, the release of these substances from the nerve endings has been demonstrated to be highly Ca^{++}-dependent[20]. A similar Ca^{++}-dependence for the release of SRIF from the cortical slices has been examined in the present study and the results are shown in figures 2 and 3. Thus, the rat cortical slices incubated in 2 ml Krebs medium containing 5.6 mm KCl and 1.5 mm $CaCl_2$ released the immunoreactive SRIF at a rate of 2.48±0.6 ng/100 mg dry wt during a 15 min incubation period. Upon raising the concentration of K^+ from 5.6 to 56.6 mM in the incubation medium (retaining isosmolarity through an equimolar reduction of NaCl), the

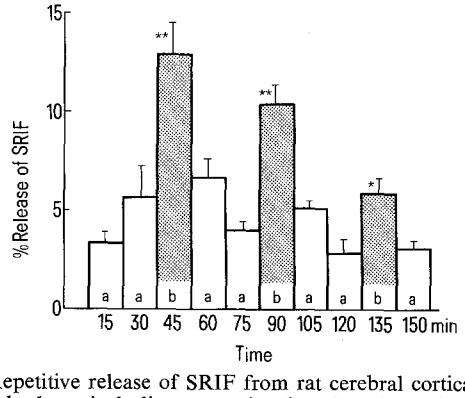

Fig. 1. Repetitive release of SRIF from rat cerebral cortical tissue. Rat cerebral cortical slices were incubated at 37 °C in 2 ml of oxygenated Krebs-bicarbonate solution containing 5.6 mM KCl and 124 mM NaCl (a), or 56.6 mM KCl and 77 mM NaCl (b). Other ingredients for both solutions were (in mM): $NaHCO_3$ 26; KH_2PO_4, 0.6; $MgSO_4$, 2.65; $CaCl_2$, 1.5; glucose, 10; pH 7.4. For each brain preparation the sum of SRIF present in all incubation media and in the tissue upon completion of incubation was taken as the total SRIF. The rate of SRIF release was calculated by expressing the amount of SRIF in 2 ml medium during the 15 min interval as percentage of the total SRIF. Each value is a mean ±SEM of 5 experiments. The statistical significance for * and ** are $p < 0.05$ and $p < 0.01$, respectively.

rate of release of SRIF was increased to 9.74±2.0 ng/100 mg dry wt 15 min ($p < 0.01$). In contrast, when the brain preparations were incubated in media in which $CaCl_2$ was replaced by 1.5 mM $MnCl_2$ the rates of SRIF release were not changed significantly upon raising the K^+ concentration from 5.6 to 56.6 mM. Also to be noted in figure 2 is that lowering the NaCl concentration from 150 to 80 mM (NaCl being replaced by choline-Cl) did not produce any significant changes in the release of SRIF.

In addition, in the synaptosome-enriched fraction isolated from rat cerebral cortex by the method described by Gray and Whittaker[21], we have found the SRIF content to be 13.2±3.1 ng/mg protein compared to 1.5±0.2 ng/mg protein and 0.33±0.01 ng/mg protein in the crude mitochondrial fraction and in the cerebral cortical homogenate, respectively. Currently, the release of SRIF from the isolated synaptosomes of rat cerebral cortex is being studied in this laboratory.

Discussion. In the present study we have demonstrated that a significant fraction of the endogenous SRIF in rat cerebral cortex is releasable upon membrane depolarization in the presence of Ca^{++}, and that the release of the peptide is likely from the nerve endings. These in vitro findings indicate that the mechanism of SRIF release indeed shares important common characteristics with other neurotransmitters and/or neuromodulators.

The functional significance and post-synaptic actions of the neocortically released somatostatin can only be derived from in vivo experiments. Iontophoretic application of somatostatin to cortical neurons in unanesthetized animals usually increases the firing rate and, furthermore, the stimulation of neuronal activity by glutamate is potentiated by the simultaneous application of somatostatin[22,23]. Stimulatory effects of somatostatin has also been observed in in vitro experiments[24], however under general anesthesia somatostatin usually reduces the firing rate in several CNS structures including cerebral cortex[25]. Supracortical[26] or intraventricular[27] administration of somatostatin significantly reduces sleep and causes restlessness, motor discoordination, and in some instances generalized tonic-clonic seizures with EEG discharges and subsequent EEG depression[8]. Intracellular recordings from cortical neurons show prolonged membrane depolarization in response to iontophoretically applied somatostatin[28]. These findings support a role for cortically - and/or CNS - released somatostatin in CNS excitatory syndromes including grand-mal type seizures.

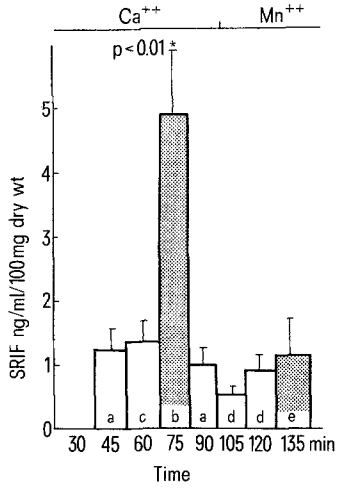

Fig. 2. Effects of Ca^{++} on the high-K^+ induced SRIF release from rat cerebral cortical tissue. Incubation procedures in general and solutions a and b are the same as in figure 1. Solution c contained 77 mM NaCl and 47 mM choline-Cl, and other ingredients as described for solution a. The Ca^{++}-deficient solutions d and e each contained 1.5 mM $MnCl_2$, while the other ingredients of these 2 solutions were the same as in solutions a and b, respectively. SRIF release is expressed as ng/ml/100 mg dry wt. (Since 2 ml of medium was used the absolute amounts of SRIF released should be 2 times as much.) Each value is a mean ±SEM of 6–8 experiments.

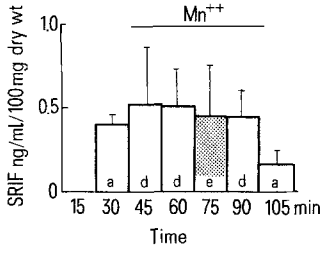

Fig. 3. Effect of Ca^{++}-deficiency on the high-K^+-induced SRIF release from rat cerebral cortical tissue. Incubation procedures and solutions are the same as described in previous figures. Each value is a mean ± SEM of 6–8 experiments.

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