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DIFFERENT RECEPTORS FOR SOMATOSTATIN AND OPIOIDS IN NEUROBLASTOMA × GLIOMA HYBRID CELLS

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1. Introduction

Neuroblastoma × glioma hybrid cells express many properties characteristic of neurons. Among these is their response to various neurohormones. Prostaglandin E_1 (PGE_1) increases the intracellular level of adenosine 3′,5′-cyclic monophosphate (cyclic AMP) in the hybrid cells. This increase is inhibited by opioids, cholinergic and adrenergic agonists (for review see ref. [1–3]). Here we report that also the tetradecapeptide somatostatin (somatotropin release inhibitory factor, SRIF) [4] blocks the raise in the level of cyclic AMP caused by PGE_1. First discovered in hypothalamus [5], SRIF occurs also in other parts of the brain [6], in the pancreas [7], stomach [7,8] and the gut [9]. It inhibits the release of somatotropin [5] and other hormones such as glucagon, insulin and gastrin [10,11]. Also the release of somatotropin evoked by morphine [12,13] is inhibited by SRIF [13]. Therefore it was hypothesized that SRIF might act as an antagonist of morphine by occupying the same receptor. Experimental support for this view was given [14]. On the other hand it has been reported that SRIF applied cerebroventricularly causes analgesia like morphine does [15].

Evidence is presented here that in the hybrid cells the specific opioid antagonist naloxone does not inhibit the action of SRIF and that SRIF does not block the specific binding of [^{3}H]naloxone to the opioid receptors of the hybird cells. It is concluded that the receptors for opioids and SRIF are different entities.

2. Materials and methods

Somatostatin preparations from the following sources were used: UCB Bioproducts, Brussels; Beckman Instruments, Geneva; and Kabi, Stockholm (gift from Dr A. Wahlström). Leucine-enkephalin was a gift Drs L. Moroder and E. Wünsch, Martinsried; PGE_1 from Dr J. Pike, Upjohn Co., Kalamazoo, Michigan; etorphine from A. Herz, Munich; naloxone·HCl and R020-1724 from Hoffmann-LaRoche, Grenzach, FRG. [^{3}H]Naloxone (20.0 Ci/mmol) was from New England Nuclear.

Plating of the cells on to plastic Petri dishes and growth have been described previously [16]. For experimental incubation in dishes 85 mm in diameter the growth medium was removed and the cells were washed with 5 ml incubation medium [17]. Subsequently the cells were incubated with 5 ml incubation medium and the various additions at 37°C for 10 min. After the incubation the cellular concentration of cyclic AMP was determined [16].

For measuring binding of [^{3}H]naloxone to hybrid cells, 5.6–6.3 × 10^6 viable hybrid cells (viability 70%, exclusion of nigrosin) per plastic plate (150 mm in diameter) were harvested by incubation (4°C, 10 min) with medium D1 that had been adjusted [18] to 330 mOsmol with glucose and sucrose. After centrifugation the cells were resuspended in incubation buffer (50 mM Na-phosphate, 50 mM NaCl, 20 mM glucose, 100 mM sucrose, 0.8 $MgCl_2$, pH 7.4). For the determination of specific plus unspecific binding, cells containing 1 mg protein were incubated (37°C, 10 min) with 4.5 nM [^{3}H]naloxone (20.0 Ci/mmol) in incubation buffer.

For the determination of unspecific binding, 0.1 mM unlabelled naloxone was included in a parallel incubation. The difference of the data obtained in the two incubations is the specific binding. After the incubation, the mixtures were filtered by suction through Whatman GF/B filters (2.4 cm in diameter) and washed with 10 ml ice-cold incubation buffer. Before counting radioactivity, the filters were incubated for 6 h in scintillation vials containing 10 ml Rotiszint (C. Roth, Karlsruhe, FRG).

3. Results

In the hybrid cells the strong elevation in the level of cyclic AMP caused by PGE_1 is inhibited by SRIF (fig.1, curve c). Half-maximal inhibition (IC_{50}) occurs already at a concentration of 1 nM. As reported previously [16,19], also the opioid peptide leucine-enkephalin [20] inhibits the effect of PGE_1. The influence of the opioid peptide is not blocked by SRIF (fig.1, curves a and b). Rather, SRIF appears to somewhat enhance the effect of enkephalin. Thus, these results do not support the view [14] that SRIF is a partial opioid antagonist. SRIF appears to act by inhibiting the adenylate cyclase activity of the hybrid cells. During an incubation (10 min) with 0.5 mM phosphodiesterase inhibitor R020-1724 (ref. [21]) the intracellular level of cyclic AMP increased from 5–535 ± 70 pmol/mg protein. If 0.1 μM SRIF was also present, a reduced value 290 ± 1 pmol/mg protein was obtained. The result indicates that SRIF can even lower the basal rate of formation of cyclic AMP.

Neither phentolamine, a blocker of α-adrenergic receptors, nor atropin, a blocker of muscarinic cholinergic receptors, prevent the effect of SRIF (data not shown). This eliminates the possibility that SRIF acts by occupying one of these receptors also present on the hybrid cells (2,3]. If SRIF would act via opioid receptors, its action should be blocked by the specific opioid antagonist naloxone. Although naloxone reverses the inhibitory effect of leucine-enkephalin (fig. 2, curve b), it does not suppress that of SRIF (fig.2, curve a).

These results are corroborated by studies of the specific binding of [^{3}H]naloxone to the opioid receptors of intact hybrid cells. While the morphine congener etorphine competes effectively with labelled naloxone for the specific opioid binding sites (fig.3, curve a), SRIF is unable to do so (fig.3, curve b). The SRIF was still active at the end of the binding assay. This was demonstrated by the fact that it was still able to inhibit the increase in the level of cyclic AMP caused by PGE_1 (assay of the type shown in fig.1).

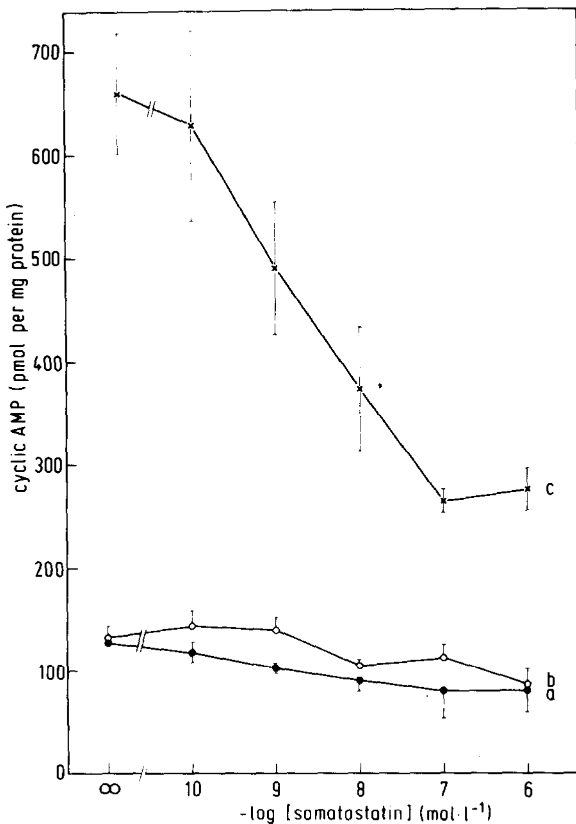

Fig.1. SRIF inhibits the elevation by PGE_1 (0.3 μM) of the level of cyclic AMP in the neuroblastoma × glioma hybrid line 108CC15 (curve c). It does not antagonize the block by 10 nM (curve a) or 100 nM (curve b) leucine-enkephalin of such an elevation. 2.5 × 10^6 viable cells/plate, 85 mm in diameter, 98% viability, passage number 17. Each value is the mean ± S. D. of data obtained from three parallel incubations. Basal level of cyclic AMP in the absence of additions: 14 ± 1 pmol/mg protein. The SRIF used here was a product of Beckman Instruments. Practically identical results were obtained with SRIF produced by Kabi or UCB Bioproducts.

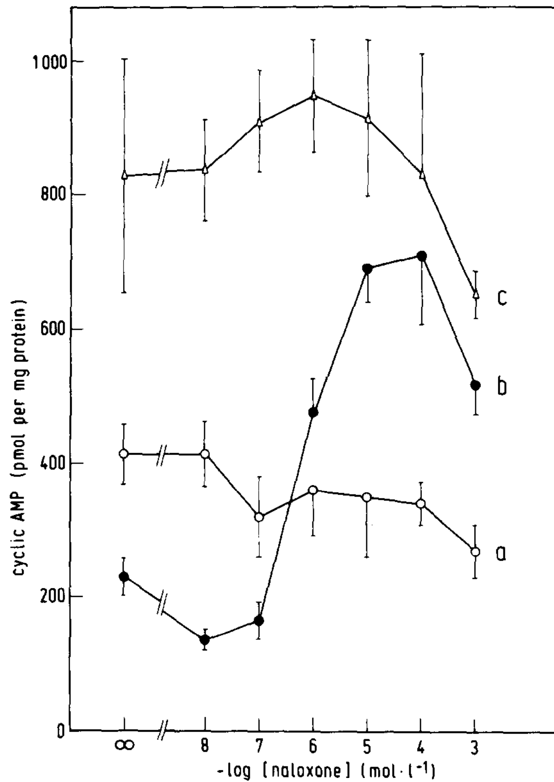

Fig.2. Naloxone prevents the inhibition by leucine-enkephalin but not by SRIF of the PGE_1-induced increase in the level of cyclic AMP. (Curve a) 0.3 μM PGE_1 + 0.1 μM SRIF; (Curve b) PGE_1 + 0.1 μM leucine-enkephalin; (Curve c) PGE_1 1.8 × 10^6 viable 108CC15 cells per plate, viability 90%, passage number 21. Basal level of cyclic AMP: 15 ± 2 pmol/mg protein. Other details as in fig.1.

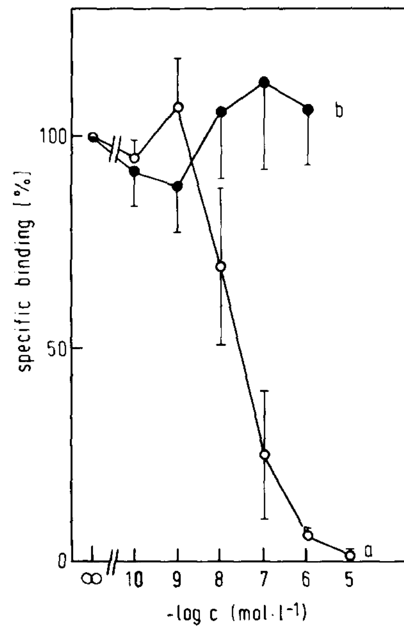

Fig.3. The morphine congener etorphine (curve a), but not SRIF (curve b), competes with [^{3}H]naloxone for the specific opioid binding sites of intact hybrid cells 108CC15. The data of curves a and b are mean values ± S. D. of mean values (triplicates) from three and four independent experiments respectively. The SRIF was from UCB Bioproducts. 5.6–6.3 × 10^6 viable hybrid cells (viability 70%) per plastic plate 150 mm in diameter were harvested as described under Materials and methods. Range of passage numbers: 16–19.

4. Discussion

SRIF has previously been shown to prevent the increase in the level of cyclic AMP that was elicited when rat anterior pituitary was incubated with PGE_1 [22–26], thyroliberin [24] or a cyclic nucleotide phosphodiesterase inhibitor [27], when rat pancreatic islets were exposed to glucose [28] or when rat hepatocytes [29] or their membranes [30] were incubated with glucagon. In congruence with these findings is the observation that the inhibition by SRIF of acid secretion from gastric mucosa is overcome by the dibutyryl derivative of cyclic AMP [31]. The present work is in accord with these reports. It provides evidence that the neuroblastoma × glioma hybrid cells carry receptors for SRIF that are not identical with those for opioids or cholinergic or adrenergic agonists. Recently, it was reported that the release of noradrenaline from a human neuroblastoma line could be blocked by SRIF [32]. In analogy the hybrid cells may be used for studying the regulation of the release of acetylcholine, a neurotransmitter they synthesize (K. Kürzinger, unpublished). Since the hybrid cells can be cultured in large quantities or be grown as tumors in mice [33], they may be a useful source for the isolation of the SRIF receptors. The absence of other cell types and the simplicity of the culture technique make the hybrid cells an attractive system for studying the mechanism of action of SRIF.

The analogy in the short-term effects on the hybrid cells of SRIF, opioids, cholinergic and adrenergic agonists suggests a common mechanism of action of the

complexes which these compounds form with their respective receptors. It is also expected that SRIF causes long-term effects similar to those of the three other classes of compounds. Such effects have been interpreted as biochemical correlates of opiate tolerance, dependence and withdrawal phenomena [2,3].

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