MORPHINE ANTAGONIZES THE ACTION OF PROSTAGLANDIN IN NEUROBLASTOMA CELLS BUT NOT OF PROSTAGLANDIN AND NORADRENALINE IN GLIOMA AND GLIOMA × FIBROBLAST HYBRID CELLS

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1. Introduction

The contraction of intestinal muscle evoked by prostaglandin E (PGE) can be inhibited by morphine [1–3]. Experiments from 2 laboratories using different systems demonstrate that morphine may exert its effect by interfering with the stimulation by PGE_1 of the adenylate cyclase system [4–6]. Thus, morphine inhibits the stimulation by PGE_1 of the formation of adenosine 3′ : 5′-cyclic monophosphate (cyclic AMP) in rat brain homogenates [4] and in mouse neuroblastoma and mouse neuroblastoma × rat glioma hybrid cells [5,6]. The specificity of the morphine actions is demonstrated by the observation that the inhibitory action of morphine can be prevented by naloxone [4,6], a morphine analog, known to antagonize the action of morphine in vivo [7]. In an extension of the studies mentioned above [4,6], the work presented here was undertaken in order to answer the questions: 1) does morphine interfere with the stimulatory effect of PGE_1 by competing with PGE_1 for the same receptor site? 2) Does morphine exert its inhibitory action on all cells in which PGE_1 stimulates the formation of cyclic AMP? 3) Is the inhibitory effect of morphine also observed in cells in which noradrenaline (NA) is known to increase the intracellular levels of cyclic AMP? The concentration of cyclic AMP in the 3 cell types used for their study is enhanced by different neurohormones: By PGE_1 in a mouse neuroblastoma line [8,9], by PGE_1 [10] or NA [11,12] in a rat glioma line and by PGE_1 or NA in a rat glioma × mouse fibroblast line [10].

Our results show that the inhibitory action of morphine cannot be overcome by high concentrations of PGE_1, thus demonstrating that PGE_1 and morphine must act at different receptor sites. Furtheron, the inhibitory effect of morphine is not found in all cell lines, in which PGE_1 stimulates the formation of cyclic AMP; and it is not observed in 2 cell lines the cyclic AMP level of which is increased in the presence of NA. The results indicate that the antagonistic action of morphine may be restricted as to the cell type and hormone affected. This study provides further evidence for the usefulness of cell cultures as model systems for studying drug action.

2. Methods

2.1. *Cell culture*

All cells used are clonal lines. Line N4TG3 is a 6-thioguanine resistant mutant [13] of line N4 [14] derived from mouse neuroblastoma C-1300, C6-BU-1 a bromodeoxyuridine resistant line [13] derived from rat glioma line C6 [15]. 54SCC11 is a hybrid line obtained by fusion of C6-BU-1 and the 6-thioguanine resistant mouse fibroblast line A9 [10,16]. The cells are routinely cultured in plastic petri dishes 10 cm in diameter (Greiner, Nürtingen) [10].

2.2. *Experimental incubation*

The experimental incubation is essentially as described [17]. Briefly, after removal of the growth medium, the cells are washed once with 5 ml incubation medium [17]. Subsequently, 5 ml of incubation medium (37°C, pH 7.4) is added, followed by 50 µl 1 mM PGE_1 in 96% ethanol and 50 µl of Dulbecco's modified Eagle's medium containing varying concen-

trations of morphine hydrochloride (Boehringer, Ingelheim). The controls receive the same volumes of pure solvents. The plates are incubated for 10 min at 37°C as described [17]. At the end of the incubation, medium and cells are separately assayed for cyclic AMP [17] using a protein binding assay [18]. In all cases, the amount of cyclic AMP released from the cells into the medium during experimental incubation was less than 10% of the total cyclic AMP in cells plus medium. Therefore, all data given are only those for the intracellular content of cyclic AMP.

3. Results and discussion

As reported [6], in neuroblastoma and neuroblastoma × glioma hybrid cells morphine inhibits the increase of intracellular cyclic AMP observed in the presence of PGE_1. In order to answer the question whether or not PGE_1 and morphine compete for the same receptor site, neuroblastoma cells were incubated with increasing concentrations of PGE_1 in the presence or absence of morphine. Fig. 1 illustrates that even at concentrations of 30 μM (more than 300 times the dose at which PGE_1 elevates cyclic AMP to half maximal value) PGE_1 does not reverse the effect of morphine. Thus, the data are suggestive of a non-competitive type of inhibition and consequently of different binding sites for PGE_1 and morphine. This result is in contrast to the report by Collier and Roy [4], who found decreased inhibition by morphine with increased PGE_1 concentration. However, the discrepancy may be only apparent, since their system is rather insensitive to PGE_1. From their data one can calculate that it is at PGE_1 concentrations between 8.8 and 88 μM that they find a major increase in formation of cyclic AMP with increased concentration of PGE_1. Now, when they increase the PGE_1 concentration from 8.8 to 177 μM in the presence of morphine they find a mean reduction of the inhibition caused by morphine from 39 to 18%. This is what one would expect for a system of noncompetitive inhibition, if one enhances the reaction rate by increasing the

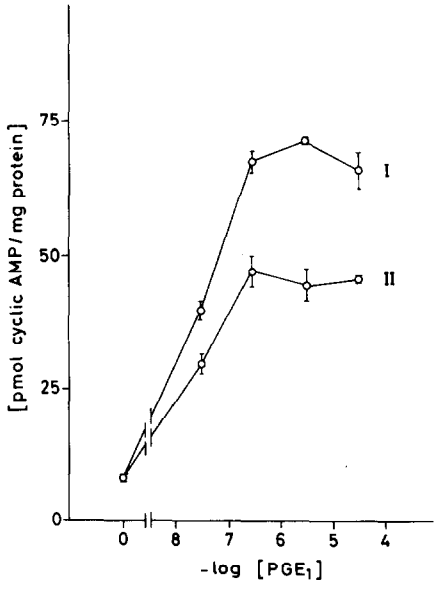

Fig. 1. Formation of cyclic AMP in presence of PGE_1 or PGE_1 and morphine as a function of the concentration of PGE_1. 19.4 million viable N4TG3 cells per plate, viability 99%; passage number 37; I: Absence of morphine; II: Presence of 30 μM morphine.

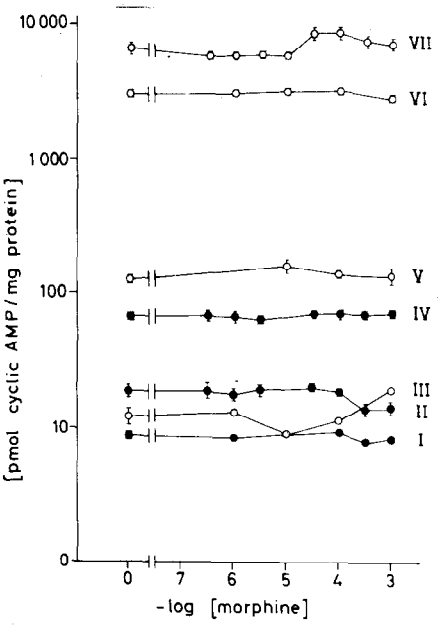

Fig. 2. Morphine and the stimulation by PGE_1 and NA of intracellular cyclic AMP in glioma line C6-BU-1 and glioma × fibroblast hybrid line 54SCC11. C6-BU-1: Passage 31, 51.4 million viable cells per plate, viability 100%. 54SCC11: passage 6, 6 million viable cells per plate, viability 94%. I: 54SCC11, no hormones; II: 54SCC11, 0.1 mM NA; III: C6-BU-1, no hormones; IV: 54SCC11, 3 μM PGE_1; V: C6-BU-1, 10 μM PGE_1; VI and VII: C6-BU-1, 1 and 100 μM NA, respectively.

saturation of receptors with agonist molecules, while keeping the concentration of antagonist constant. Inserting their data [4] into the formulae for non-competitive inhibition [19], one can compute a curve resembling the one in fig. 2 of ref. [4]. The low sensitivity to PGE_1 of the brain homogenates [4] reflects probably a damaging influence of the homogenization procedure on the PGE_1 receptors.

In most respects, rat glioma line C6-BU-1 [13] behaves very similar to the wild type line C6 [15]. Both, C6 [11] and C6-BU-1 [10,12] increase their intracellular levels of cyclic AMP in the presence of NA. C6-BU-1 does so also when exposed to PGE_1 [10]. Similarly, the clonal hybrid line 54SCC11, obtained by fusion of C6-BU-1 with mutant mouse fibroblast line A9, is sensitive to both NA and PGE_1 [10]. Thus, C6-BU-1 and 54SCC11 cells are well suited to serve in finding answers to the following questions: 1) does morphine only antagonizes the action of PGE_1 in cells of neuronal origin or character? 2)Does it also antagonize neurohormones other than PGE_1 that stimulate formation of cyclic AMP? Morphine alone does not appreciably affect the levels of cyclic AMP in lines C6-BU-1 and 54SCC11 (fig. 2, curves I and III). It does neither antagonize the stimulatory action of PGE_1 on 54SCC11 (curve IV) and C6-BU-1 (curve V) nor of NA on 54SCC11 (curve II) and C6-BU-1 (curves VI and VII). That the stimulation of these cells by NA is susceptible to inhibition by a suitable substance is demonstrated in table 1. In both cell lines propranolol, a β-adrenergic blocking agent, completely prevents the increase of the intracellular level of cyclic AMP evoked by NA. Thus, in both cell lines neither the stimulation of cyclic AMP formation by PGE_1 nor that by NA is antagonized by morphine. These resulfs demonstrate that morphine does not interfere with the action of PGE_1 on all types of cells. In fact, the data suggest that susceptibility to morphine exists only in certain cell types and that nerve cells, but probably not glial cells, may be among them. However, our data do not contribute to answering the questions: a) are all nerve cells sensitive to PGE_1 also susceptible to morphine? b) Are all glial cells sensitive to PGE_1 insensitive to morphine? c) Are there cells, in which morphine antagonizes the stimulatory effect of NA or other hormones known to act via cyclic nucleotides as second messengers? In other words is PGE_1 the only hormone antagonized by morphine? Are there cases, in which prostaglandins other than PGE_1 are antagonized by the narcotic? It is tempting to assume that cells insensitive to morphine are lacking an 'opiate receptor'. Presently we are testing this hypothesis. Question a) might be answered tentatively. While opiate receptors are abundant in the limbic system, they are hardly detectable in other areas of the brain [20–22]. Provided that nerve cells in regions devoid of opiate receptors are responsive to PGE_1, question a) must be answered negatively.

Table 1
The β-adrenergic antagonist propranolol inhibits the increase of the concentration of intracellular cyclic AMP elicited by NA. Glioma cells and glioma × fibroblast hybrid cells are studied. The experimental incubations are analogous to those described in Methods.

	concentration of additions (μM)		cyclic AMP
cell line	NA	propranolol	(pmol/mg protein)
glioma C6-BU-1	0	0	12 ± 2*
	0	200	10.0 ± 1.7
	1	0	3100 ± 40
	100	0	6500 ± 600
	100	200	13.2 ± 1.6
glioma × fibroblast	0	0	9.3 ± 2.1
hybrid 54SCC11	0	100	10.0 ± 1.0
	100	0	19.3 ± 2.2
	100	100	8.9 ± 0.7

* Standard deviation

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