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MORPHINE ANTAGONIZES THE POSITIVE CHRONOTROPIC EFFECT OF PROSTAGLANDINS IN GUINEA PIG ATRIA BUT NOT THE INCREASE IN CYCLIC AMP CONTENT*

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Abstract—Prostaglandins E_1 and E_2 increase the frequency of the spontaneously beating isolated guinea pig atria. An ED_{50} of 6×10^{-10} M is obtained for both prostaglandins. Morphine, at 10^{-4} M, inhibits the prostaglandin-stimulated increase in frequency in a noncompetitive manner. PGE_1 but not PGE_2 elevates cAMP level about 33 per cent above controls. This increase in cAMP content is not antagonized by morphine. This data provide evidence that the inhibitory effect of morphine on the prostaglandin stimulated frequency does not involve the cAMP generating system.

Prostaglandins are implicated in a wide variety of biological processes such as muscle contraction, reproduction, kidney function, gastric acid secretion and nervous activity. Cellular action of prostaglandins is considered to be closely related to changes in the activity of the cAMP generating system [1, 2, 4]. However, the precise mode of action of prostaglandins at the molecular level is still a matter of much speculation. Recently, it has been suggested that morphine may antagonize prostaglandin-stimulated cAMP formation [5, 6]. Since it had been shown that prostaglandins of the E series have a positive chronotropic effect in the spontaneously beating atria from guinea pig [3], it was of interest to see whether this effect is associated with a concomitant increase in intracellular cAMP concentration. Our results show that PGE_1 stimulates both, cAMP formation and the frequency of beating of atria, but morphine antagonizes the latter effect only, having no effect on the prostaglandin-stimulated increase in cAMP levels.

MATERIALS AND METHODS

Male guinea pigs (200–250 g) were killed by a blow on the neck, the heart was excised immediately and placed in oxygenated Krebs–Henseleit solution. The right and left atria were dissected from the rest of the heart and mounted in a 10-ml organ bath containing Krebs–Henseleit solution with 15 mM glucose. The incubation medium was continuously bubbled with 95% O_2 and 5% CO_2 and maintained at 30°.

The atria (50–70 mg wet wt.) were allowed to beat spontaneously. Resting tension was adjusted to 0.5 g and maintained at that level over the period of the experiment. The frequency was recorded by a ratemeter measuring the reciprocal value of the distance between two contraction cycles. In all experiments atria were equilibrated for 60 min in the incubation medium before drugs were added.

Cumulative dose–response curves for the chronotropic effects were established using the 1/2 log 10 interval as described by van Rossum [7]. Each preparation was used only for one dose–response curve. The results are expressed as rise in frequency above the unstimulated level. After total incubation time of 2 hr the atria were rapidly weighed and homogenized in a ground-glass homogenizer containing 1 ml 0.4 N perchloric acid. cAMP was purified using Dowex 1 × 8 columns as described earlier [8]. Determination of cAMP was carried out using the method described by Gilman [15], each sample being analyzed at three different concentrations. The data were corrected for recovery and are expressed as pmoles cAMP/100 mg tissue wet wt. Statistical evaluation of results as performed using Student's *t*-test.

Materials. Prostaglandins were a gift from Dr. J. Pike, Upjohn Comp., Kalamazoo, USA, all other chemicals were from usual commercial sources.

RESULTS

PGE_1 and PGE_2 increase the frequency of the beating atria in a dose-dependent manner (Fig. 1). The efficacy of both prostaglandins is almost identical, an ED_{50} of 6×10^{-10} M is obtained for both compounds. The increase in frequency cannot be antagonized by pindolol, a β-adrenoceptor blocking agent. Also, pretreatment with reserpine does not prevent the positive chronotropic effect of prostaglandins. This indicates that this effect is not mediated vai stimulation of adrenergic β-receptors [9]. However, in the presence of 10^{-4} M morphine the positive chronotropic response to PGE_1 and PGE_2 is reduced by about 50 per cent (Fig. 1). The change in the shape of the dose–response curve to the prostaglandins shows that morphine acts as a noncompetitive inhibitor. Morphine alone at 10^{-4} M concentration has no

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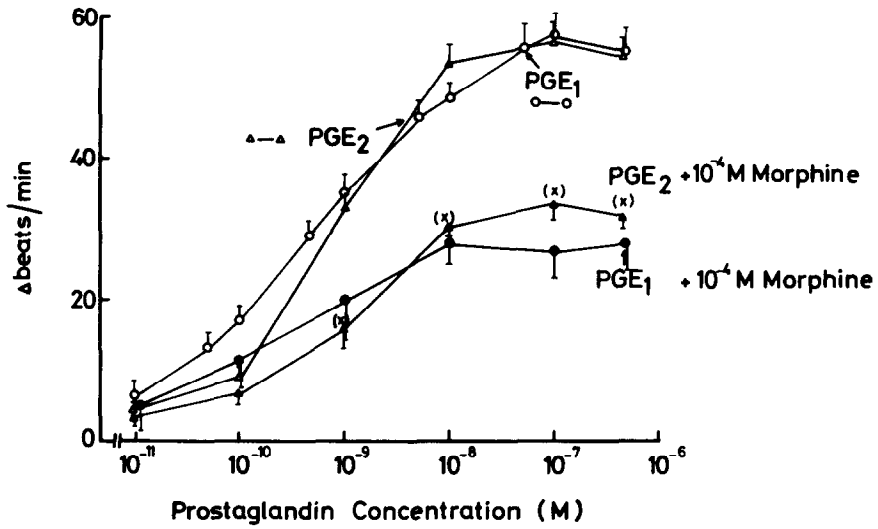

Fig. 1. Effect of 10^{-4} M morphine on prostaglandin E_1 and E_2 stimulated frequency of the isolated guinea pig atria. The ordinates express the changes in the beats per min above the unstimulated level (126 ± 4 beats/min). The results are given as the mean ± S.E.M. of five separate experiments (×): $P < 0.005$, PGE_1 or PGE_2 with morphine compared with PGE_1 or PGE_2 without morphine.

effect on the frequency of beating of atria. Interestingly, we observed that morphine had to be added to the incubation medium before the addition of prostaglandins in order to see its inhibitory effect. An addition of morphine, after the maximum response of PG was reached, has no effect on the increased frequency, at all. When cAMP levels were determined in the atria, no correlation with the positive chronotrophic effect of PGE_1 or PGE_2 could be observed. cAMP levels were increased significantly by PGE_1 but not by PGE_2 (Table 1). The same dose of morphine which significantly reduced the increase in frequency, did not block the PGE_1-enhanced cAMP accumulation (Table 1).

DISCUSSION

Our results show a distinct discrepancy between the effect of PGE_1 and PGE_2 on the cAMP generating system and the frequency of the spontaneously beating guinea pig atria. Since both prostaglandins used in this study are about equipotent in increasing the beating rate one may speculate that they interact with the same receptor site in the atria. This binding site has about equal affinity for PGE_1 and PGE_2. The inhibition of this effect by morphine suggests that morphine may act at either the same or a closely related site to the prostaglandin receptors. An opiate receptor has been well-characterized in brain and in a hybrid cell line obtained from neuroblastoma and glioma cells [10,11]. It is conceivable that such an opiate binding site exists in the atria as well and is the site of action for the morphine effect on the frequency.

Only PGE_1 elicited a significant increase in intracellular cAMP, while PGE_2 showed only a negligable effect. This observation confirms other experiments in which cultured myocardial cells were used [3]. The dissimilar effect of PGE_1 and PGE_2 in the cAMP generating system compared to their equal potency as positive chronotropic agents may be an indication that the receptor sites involved in cAMP formation and those linked to the frequency increase are not identical. Also, the data seem to suggest that the increase in cAMP has no direct correlation with the positive chronotropic effect of the prostaglandins since morphine inhibits only the latter.

Recently, several hypothesis have been proposed concerning the molecular basis for narcotic action [5, 14]. It was suggested that morphine acts either by inhibiting the prostaglandin-elicited cAMP formation in the CNS or directly inhibits the adenylate cyclase. Compensatory shifts of the enzymes involved in

Table 1. Effect of prostaglandins and morphine on cAMP levels in isolated guinea pig atria

	Cyclic AMP (pmoles/100 mg wet wt ± S.E.M.)	
Control	171.1 ± 9.3	(17)
PGE_1 5×10^{-7} M	255.5 ± 16*	(8)
PGE_2 5×10^{-7} M	182.9 ± 9.9	(7)
Morphine 10^{-4} M + PGE_1 5×10^{-7} M	274.5 + 11*	(6)
Morphine 10^{-4} M + PGE_2 5×10^{-7} M	190.5 + 8.1	(5)

Number of individual experiments in parentheses.
* $P < 0.005$ compared with control.

the regulation of cAMP would finally be responsible for the development of tolerance and addition. Our results in guinea pig atria support earlier observations on antagonism between prostaglandin E_1 and morphine in guinea pig ileum [6] and in human blood platelets [12], but it seems doubtful whether the cAMP generating system is a necessary requisit for this antagonism in all systems.

Recently it was shown that not only morphine but also acetylcholine increase cGMP levels in a neuroblastoma glioma hybrid cell line [13]. It seems an interesting question whether the action of morphine in guinea pig atria involves an increase in cGMP.

REFERENCES

1. P. Needleman, M. S. Minkes and J. R. Douglas Jr., *Circulation Res.* **34**, 455 (1974).
2. R. W. Butcher and C. E. Baird, *J. biol. Chem.* **243**, 1713 (1968).
3. A.-M. Moura and H. Simpkins, *J. molec. Cell. Cardiol.* **7**, 71 (1975).
4. A. Wollenberger, E. B. Balskii, E. G. Krause, S. Genz, D. Blohm and E. v. Bogdanova, *Biochem. biophys. Res. Commun.* **55**, 446 (1973).
5. H. O. J. Collier and A. C. Roy, *Prostaglandins* **7**, 361 (1974).
6. R. Jaques, *Experientia* **25**, 1059 (1969).
7. J. M. van Rossum, *Archs int. Pharmacodyn.* **143**, 299 (1963).
8. J. Schultz and J. W. Daly, *J. biol. Chem.* **248**, 843 (1973).
9. J. Borbola, K. Süsskand, M. Siess and L. Szekeres, *Eur. J. Pharmac.*, in press.
10. H. O. J. Collier and A. C. Roy, *Nature* **248** 24 (1974).
11. J. Traber, K. Fischer, S. Latzin and B. Hamprecht, *FEBS* **49**, 260 (1974).
12. R. J. Gryglewski, A. Szczeklik and K. Bieron, *Nature* **256**, 56 (1975).
13. R. Gullis, J. Traber and B. Hamprecht, *Nature* **256**, 57 (1975).
14. S. K. Sharma, W. A. Klee and M. Nirenberg, *Proc. natn. Acad. Sci. U.S.A.* **72**, 3092 (1975).
15. A. G. Gilman, *Proc. natn. Acad. Sci., Wash.* **67**, 305 (1970).