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Methylxanthines antagonize adenosine but not morphine inhibition in guinea pig ileum[1]

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In the electrically stimulated guinea pig ileum longitudinal muscle strip preparation the inhibitory actions of adenosine were antagonized by various concentrations of theophylline (12.5 and 100 μM) and 8-phenyltheophylline (4 and 20 μM). However, the inhibitory actions of morphine were not antagonized by the same concentration of theophylline and 8-phenyltheophylline. These results suggest that in the guinea pig ileum longitudinal muscle strip adenosine is not a mediator in the inhibitory actions of morphine.

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On a neutralisé l'action inhibitrice de l'adénosine dans la préparation d'un segment du muscle longitudinal de l'iléon de cobaye stimulée par voie électrique et ce, à l'aide de différentes concentrations de théophylline (12,5 et 100 μM) et de phénylthéophylline (4 et 20 μM). Toutefois, l'action inhibitrice de la morphine ne fut pas neutralisée par une même concentration de théophylline et de 8-phénylthéophyline. Ces résultats suggèrent que, dans le segment du muscle longitudinal de l'iléon de cobaye, l'adénosine n'est pas un médiateur de l'action inhibitrice de la morphine.

[Traduit par le journal]

Introduction

Previous reports have shown that theophylline antagonized the inhibitory actions of morphine in the guinea pig ileum longitudinal muscle strip (GPI-LMS) (Sawynok and Jhamandas 1976, 1979; Jhamandas and Sawynok 1976) and rat brain (Jhamandas *et al.* 1978; Stone and Perkins 1979; Perkins and Stone 1980). Stone and Perkins (1979) hypothesized that morphine inhibition was due to a local release of adenosine following interaction of morphine with the opiate receptor. This concept has been supported by recent evidence that morphine enhanced (1) the veratridine-induced release of purines (Fredholm and Vernet 1978) and (2) the release of purines from intact rat cerebral cortex (Phillis *et al.* 1980). Perkins and Stone (1980) proposed that in the rat cortex theophylline antagonism of morphine inhibition was due to antagonism of the action of the adenosine which was released by morphine.

The purpose of this study was to determine the significance of adenosine release in morphine inhibition in the electrically stimulated GPI-LMS preparation. This was done by comparing the effect of various concentrations of theophylline and 8-phenyltheophylline, a potent and specific adenosine antagonist (Smellie *et al.* 1979), on adenosine and morphine dose–response curves.

Methods

Guinea pig ileum longitudinal muscle strip preparation

The preparation of the GPI-LMS was similar to that of Paton and Vizi (1969) as described by Clement (1980). Cumulative dose–response curves were constructed for morphine and adenosine. The twitch height following addition of drug was expressed as a percentage of the control twitch height. Theophylline or 8-phenyltheophylline was added to the organ bath, containing Krebs–Henseleit solution at 37°C, 10 min before running the cumulative dose–response curve.

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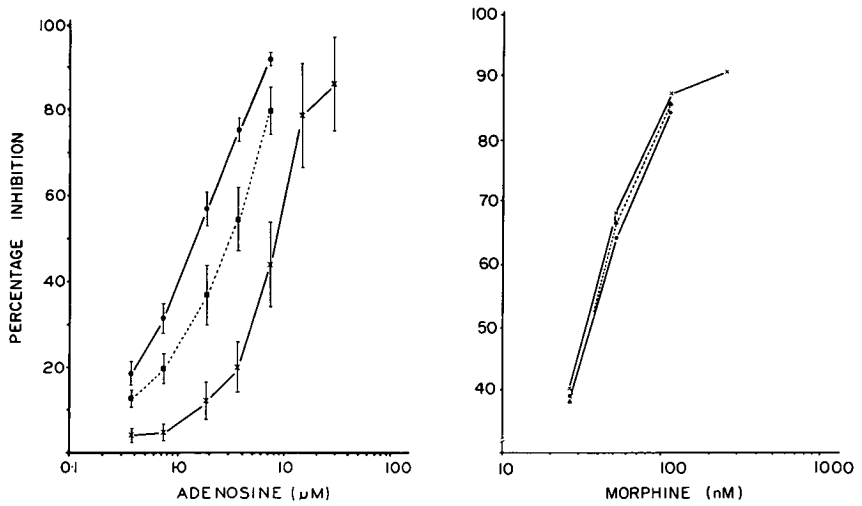

FIG. 1. Effect of various concentrations of theophylline on the adenosine and morphine dose–response curves in the GPI-LMS. ●, Control; ■, theophylline (12.5 μ*M*); ▲, theophylline (25 μ*M*); ×, theophylline (100 μ*M*). Each point represents the mean ± SEM of at least six separate observations.

The IC_{50} concentrations and the 95% confidence limits were determined by the method of Litchfield and Wilcoxon (1949).

Drugs used

Drugs were morphine sulfate (BDH), adenosine (Boehringer-Mannheim), theophylline (Sigma), and 8-phenyltheophylline (Calbiochem-Behring). The 8-phenyltheophylline was dissolved in dimethyl sulfoxide and an aliquot (5 or 25 μL) was added directly to the organ bath.

Results

Theophylline antagonism of morphine and adenosine

The results in Fig. 1 demonstrate that various concentrations of theophylline (12.5 and 100 μ*M*) antagonized the adenosine inhibition of the twitch response in GPI-LMS. Theophylline (100 μ*M*) increased the adenosine IC_{50} from a control value of 1.45 μ*M* (0.7–2.8 μ*M*; 95% confidence limits) to 7.8 μ*M* (4.9–12.5 μ*M*) whereas theophylline (12.5 μ*M*) only increased the adenosine IC_{50} to 2.35 μ*M* (1.4–4.7 μ*M*). In contrast with the results obtained with adenosine, theophylline *did not* antagonize the morphine inhibition of the twitch response (Fig. 1). Theophylline (12.5 μ*M*–100 μ*M*) augmented the twitch response in control preparations 10%.

8-Phenyltheophylline antagonism of morphine and adenosine

8-Phenyltheophylline specifically antagonized the inhibition of twitch response by adenosine in a dose-related manner (Fig. 2). A low concentration of 8-phenyltheophylline (4 μ*M*) increased the adenosine IC_{50} from a control value of 1.8 μ*M* (0.9–3.6 μ*M*; 95% confidence limits) to 6.2 μ*M* (3.7–10.3 μ*M*) whereas a higher concentration (20 μ*M*) increased the adenosine IC_{50} to 15.5 μ*M* (9.8–24.5 μ*M*).

8-Phenyltheophylline (20 μ*M*) only slightly antagonized the morphine inhibition of the twitch response in the GPI-LMS (Fig. 2). The difference was not significant.

Discussion

The results in this study demonstrate that theophylline and 8-phenyltheophylline at the concentrations used antagonize adenosine inhibition but not morphine inhibition of the twitch response in the GPI-LMS. The lack of theophylline antagonism of morphine inhibition is in agreement with the results of Gintzler and Musacchio (1975) in the guinea pig ileum. In support of our findings, recent reports showed that isobutylmethylxanthine did not antagonize morphine inhibition of individual neurons in the guinea pig ileum myenteric plexus (Karras and North 1979) or cat spinal cord (Duggan and Griersmith 1979). The earlier studies of Jhamandas and Sawynok (Jhamandas and Sawynok 1976; Sawynok and Jhamandas 1976) reported a theophylline antagonism of morphine inhibition and suggested that there was a link between adenosine and morphine inhibition in the GPI-LMS. However, the concentrations of theophylline they used were higher than those used in this study. The theophylline antagonism of morphine inhibition they observed could be due to another action of theophylline (perhaps Ca^{2+} mobilizing actions) (Sawynok and Jhamandas 1979) at the higher concentrations. In rat brain Perkins and Stone (1980) tend to discount the involvement of Ca^{2+}.

With the work of Jhamandas and Sawynok their conditions of stimulation of the morphine-treated tissues were different from the adenosine-treated tissues, i.e.,

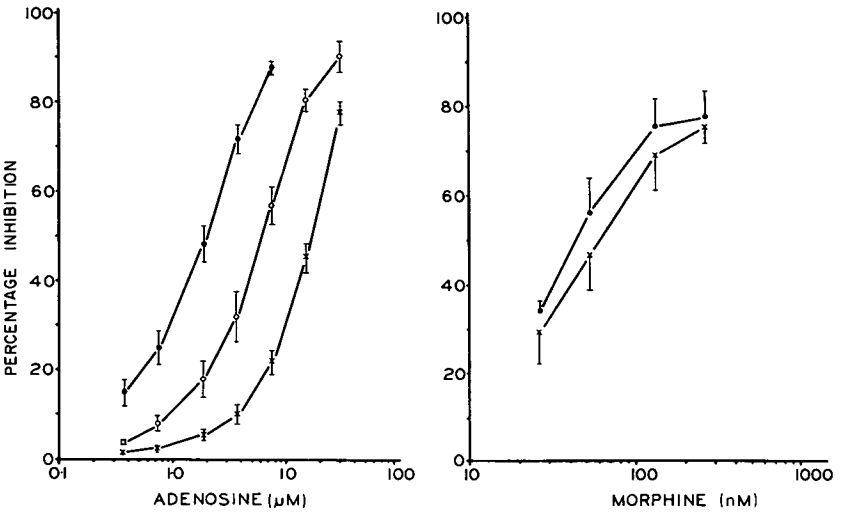

FIG. 2. Effect of various concentrations of 8-phenyltheophylline on the adenosine and morphine dose–response curves in GPI-LMS. ●, Control; ○, 8-phenyltheophylline (4 μ*M*); ×, 8-phenyltheophylline (20 μ*M*). Each point represents the mean ± SEM of at least six separate observations.

stimulus strength was 40 and 80 V, respectively. As shown in this study, theophylline (12.5–100 μ*M*) augmented the supramaximal twitch response in control preparations ~10%. Perhaps the submaximal stimulus conditions they used with morphine allowed the theophylline augmentation of the twitch response to become exaggerated, thus suggesting antagonism.

It is possible that exogenous and endogenous adenosine may differ in its ability to be antagonized by various concentrations of methylxanthines. In the biophase, morphine could produce higher concentrations of adenosine than those applied exogenously. However, this does not appear to be the case as either an adenosine deaminase inhibitor, erythro-9-(2-hydroxy-3-nonyl) adenine, or a nucleoside uptake inhibitor, 6-(2-hydroxy-5-nitro)-benzylthioguanosine, potentiated the inhibition of exogenously applied adenosine but did not potentiate the morphine inhibition (J. G. Clement, unpublished observations).

8-Phenyltheophylline, which lacks phosphodiesterase inhibiting properties at the concentrations used in this study, was a potent antagonist of adenosine inhibition in the GPI-LMS in agreement with the results of Smellie *et al.* (1979). Theophylline (100 μ*M*) increased the IC_{50} for adenosine 5.38 times where 12.5 μ*M* theophylline had no significant effect. 8-Phenyltheophylline (4 and 20 μ*M*) increased the IC_{50} for adenosine 3.44 and 8.61 times, respectively.

The results of this study suggest that adenosine is not a mediator of morphine inhibition in the GPI-LMS in agreement with the conclusions of Hayashi *et al.* (1978). However, this does not preclude the fact that adenosine may be a mediator of morphine action in the central nervous system *et al.* 1980; Stone and Perkins 1979; Perkins and Stone 1980; Fredholm and Vernet 1978).

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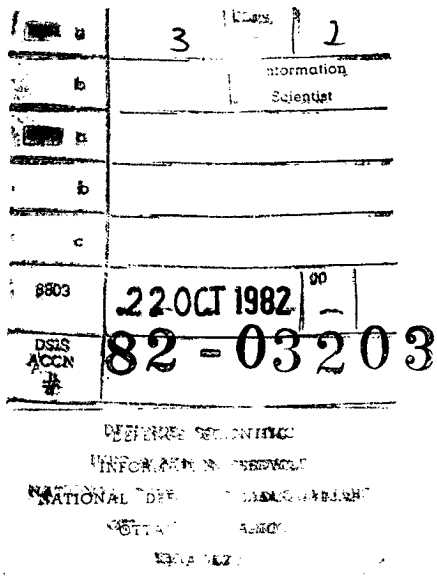
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