Br. J. Pharmac. (1981), **73**, 787–789

THEOPHYLLINE DOES NOT AFFECT MORPHINE INHIBITION OF THE ISOLATED VAS DEFERENS

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1 Adenosine and morphine both produced an inhibition of the electrically-evoked twitch of the isolated superfused vas deferens of the mouse.

2 Theophylline, 10 or 100 μM, reduced the inhibitory action of adenosine but did not change inhibition by morphine.

3 It is suggested that adenosine release is not a necessary prerequisite for morphine inhibition of transmitter release. It is also suggested that the involvement of adenosine may follow activation only of opiate μ-receptors.

Introduction

Both adenosine and morphine are potent inhibitors of neurotransmitter release in peripheral tissues (Ginsborg & Hirst, 1972; Henderson, Hughes & Kosterlitz, 1972; Henderson & Hughes, 1975; Vizi & Knoll, 1976; Clanachan, Johns & Paton, 1977; Ribeiro, 1979; Stone, 1981) as well as in the central nervous system (Jhamandas, Hron & Sutak, 1975; Lekić, 1977; Jhamandas, Sawynok & Sutak, 1978; Harms, Wardeh & Mulder, 1979).

A report by Sawynok & Jhamandas (1976) therefore aroused great interest with the demonstration that the presynaptic inhibitory effects of both adenosine and morphine on the guinea-pig myenteric plexus-longitudinal muscle preparation, could be blocked in parallel by the adenosine antagonist theophylline, whereas the inhibitory effects of adrenaline and dopamine were unaffected. The suggestion was made that the inhibitory effects of morphine in this system might involve the initial release of adenosine which would be the agent responsible for the suppression of transmitter release. A number of subsequent studies have investigated this possibility in the central nervous system, with the demonstration that morphine enhances adenosine release from central nervous tissue (Fredholm & Vernet, 1978; Phillis, Jiang, Chelack & Wu, 1979) and that morphine's effects on transmitter release (Jhamandas *et al.*, 1978) and neuronal firing (Perkins & Stone, 1980) can be blocked by methylxanthines.

However, Sawynok & Jhamandas (1976) remarked on the relative insensitivity of their ileal preparation to morphine. In the present paper the effect of theophylline has been examined on morphine inhibition of the mouse vas deferens, as this is one of the tissues which is most sensitive to the presynaptic inhibitory effects of morphine (Henderson *et al.*, 1972).

Methods

Mice (TO strain) were killed by stunning and cervical dislocation and the vasa deferentia removed into cold medium of the following composition (mM): NaCl 118, KCl 4.70, $CaCl_2$ 2.48, $NaHCO_3$ 24, KH_2PO_4 0.85 and glucose 11.

Adhering connective tissue and blood vessels were removed from one vas and the tissue gently compressed to express luminal semen. The vas was transferred into a 5 ml organ bath through which solution of the above composition was perfused continuously at a rate of 3 ml per min. The solution was warmed before entering the bath so that the bath temperature was maintained between 35 and 37°C. The solution in the bath was gassed with a 95% O_2/5% CO_2 mixture.

The vas was attached to an isometric strain gauge transducer under a resting tension of approximately 0.5 g. Contractions of the vas were recorded on a Devices M4 pen recorder. Field stimulation of the vas was effected by a pair of parallel platinum wires placed on either side of the preparation, using pulses of 1–2 ms duration and supramaximal voltage (normally 80 V), delivered at 0.1 Hz.

Adenosine and morphine were added directly to the bath in volumes of 0.05 or 0.1 ml. The concentrations quoted are the estimated concentrations attained in the bath. Theophylline was added to the perfusing medium at the required concentration.

0007-1188/81/070787-03 $01.00

Results

Both morphine and adenosine produced inhibition of the electrically evoked twitch of the mouse vas (Figure 1). The ability of low concentrations of naloxone to reverse the morphine inhibition (Figure 1) confirmed the involvement of specific opiate receptors in this response. Furthermore the failure of both compounds to modify contractile responses to noradrenaline (10 μM) or ATP (10 μM) confirmed the presynaptic site of the inhibitory action.

Dose-response curves for the inhibitory effects of adenosine and morphine are shown in Figure 2. Theophylline, at 10 and 100 μM caused a significant shift to the right of the dose-response curve for adenosine, while responses to morphine were unaffected at both theophylline concentrations (Figure 2). At 10 μM, theophylline itself produced no change of contraction height, but at 100 μM the twitch height was reduced by about 20% after 30 min.

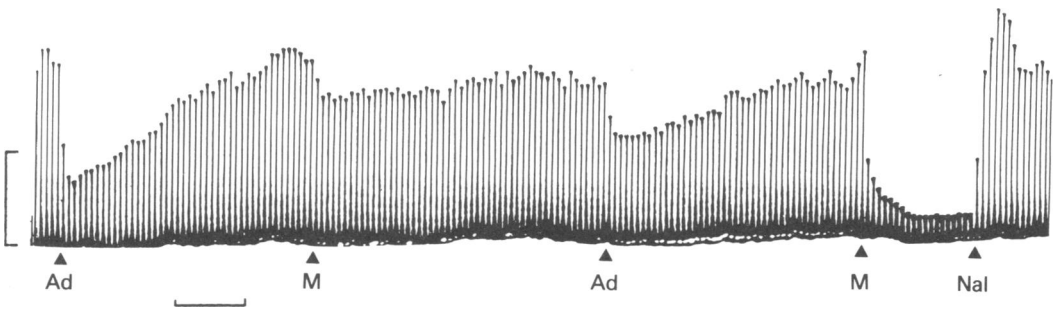

Figure 1 Chart recordings of the electrically evoked contractions of the isolated vas deferens of the mouse. The record shows the inhibitory effects of adding adenosine (Ad) 20 μM, morphine 0.5 μM (M), adenosine 5 μM and morphine 5 μM. The inhibition produced by the higher dose of morphine is shown to be reversed by naloxone (Nal) 0.2 μM. Calibrations: 200 mg tension and 2 min.

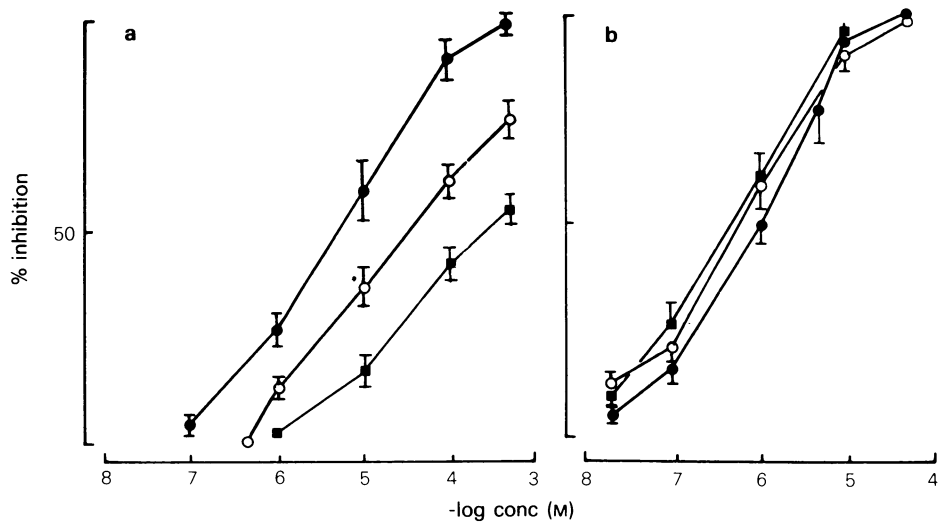

Figure 2 Dose-response curves for the inhibition of electrically-evoked contractions of the mouse vas deferens by (a) adenosine and (b) morphine. In each case curves are shown for normal perfusing medium (●) and during perfusion with theophylline 10 μM (○) and 100 μM (■). Note the shift to the right of the dose-response curve for adenosine but not morphine. Each point is the mean of at least 4 measurements; vertical lines show s.e.mean. All points on the adenosine graphs are shifted significantly by theophylline at both concentrations ($P < 0.05$, Student's t test), but there is no significant shift of any point for morphine.

Discussion

The ability of methylxanthines such as theophylline to prevent the inhibitory effect of morphine on neurotransmitter release has been noted on preparations of guinea-pig ileum (Gintzler & Musacchio, 1975; Sawynok & Jhamandas, 1976) and on tissue from the central nervous system (Jhamandas *et al.*, 1978).

It has also been reported that morphine will enhance release of adenosine from slices or synaptosomes prepared from central tissue (Fredholm & Vernet, 1978; Stone, unpublished observations) and that methylxanthines can block the depressant effects of morphine on neuronal firing (Perkins & Stone, 1980; Stone, 1981).

The importance of the present observations therefore probably lies in indicating that, in spite of all the positive evidence just quoted, morphine's inhibitory action on transmitter release is not *necessarily* mediated by an initial adenosine release. Indeed the innervation of the vas deferens is arguably much simpler than that of the ileum, or, of course, brain, and more complex interactions may explain some of the earlier observations.

Alternatively, it is interesting to note that the predominant species of opiate receptor is different in the mouse vas and guinea-pig ileum. In the former, δ-receptors predominate, whereas μ-receptors appear to predominate in ileum (Lord, Waterfield, Hughes & Kosterlitz, 1977). Conceivably then, adenosine release and mediation of morphine effects may be a characteristic of μ-receptor, but not δ-receptor activation.

A am grateful to the M.R.C. for grant support.

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(*Received February 13, 1981.*)