J. Pharm. Pharmacol. 1996, 48: 320–322
Received February 15, 1995
Accepted October 11, 1995

Glibenclamide Antagonizes the Inhibitory Effect of Morphine on Gall Bladder Emptying

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Abstract

Egg yolk-induced gall-bladder emptying in mice was used to investigate the effect of glibenclamide and minoxidil (ATP-dependent K^+-channel modulators) on biliary tract effects of morphine.

The inhibitory effect of morphine (1–4 mg kg^{-1}, i.p.) on egg yolk-induced gall-bladder emptying was completely blocked by pretreatment with naloxone (2 mg kg^{-1}, i.p.) or glibenclamide (0·65 mg kg^{-1}, i.p.) whereas, pretreatment with minoxidil (0·65 mg kg^{-1}, i.p.) did not modify the inhibitory effect of morphine on gall-bladder emptying.

Our results suggest that biliary-tract actions of morphine are mediated through glibenclamide-sensitive K^+ channels similar to those involved in the analgesic action of morphine.

Several studies have shown that ATP-dependent K^+ channels are involved in the antinociceptive effect of morphine (Ocana et al 1990; Welch & Dunlow 1993). However, there are conflicting reports on the involvement of ATP-dependent K^+ channels in the gastrointestinal effects of morphine. Bhounsule et al (1992) have reported that the protective effect of morphine on ethanol-induced gastric lesions in rats may involve ATP-dependent K^+ channels, but a recent study has shown that glibenclamide failed to antagonize the intestinal actions of morphine (Sunita et al 1994). Thus, the role of ATP-dependent K^+ channels in the gastrointestinal action of morphine is still unclear.

Acute or chronic administration of morphine has been shown to delay cholecystokinin (CCK)-induced gall bladder emptying (Worobetz et al 1982; Malave & Yim 1993). This naloxone-reversible effect was attributed to opioid-induced spasm of the sphincter of Oddi (Economou & Ward-McQuaid 1971; Malave & Yim 1993). Involvement of ATP-dependent K^+ channels in the effect of morphine on the biliary tract is lacking. In the present study, the effect of glibenclamide and minoxidil on the inhibitory effect of morphine on gall-bladder emptying is investigated to verify whether the spasmogenic effect of morphine on the biliary tract is mediated through ATP-dependent K^+ channels.

Materials and Methods

Animals

Male Swiss albino mice 20 ± 1 g (Vaccine Institute, Belgaum, India), were divided into eleven groups of ten each and were housed under standard animal room conditions (12 h light/dark cycle) with free access to food and water for at least one week before the experiment. The animals were starved for 24 h before the experiment with free access to water.

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Drugs

Morphine sulphate and naloxone hydrochloride (K.L.E.S.'s Hospital and Medical Research Centre, Belgaum) were dissolved in saline (0·9% w/v). Glibenclamide (Hoechst India) was dissolved in 5% Tween 20/saline. Minoxidil (Koppran Chemicals) was dissolved in ethanol/saline.

Biliary Motility

Biliary motility was determined by the method of Valsecchi & Toson (1982) which is based on the fact that the presence of egg yolk in the duodenum releases CCK which causes contraction and emptying of the gall bladder and reduces its weight. Thus, it is the weight of the gall bladder at death which serves as a major indication of its earlier motility. Animals of one group received saline by both oral (1 mL) and intraperitoneal (0·25 mL) routes to establish the normal weight of the gall bladder. In animals of all other groups, gall-bladder emptying was induced by oral administration of 1 mL of a 30% suspension of lyophilized egg yolk in saline, 8 min after administration of drug or saline (i.p.). Animals were killed by ether inhalation 15 min after oral administration of saline or egg yolk. Gall bladders were removed by sectioning the cystic duct and weighed. The average weight of the gall bladder was calculated for each group. Percent inhibition of emptying was calculated as:

$$\text{inhibition of emptying (\%)} = (T_i - C) \times 100/(B - C) \quad (1)$$

where B = mean weight (mg) of gall bladder in saline group, C = mean weight (mg) of gall bladder in the egg-yolk (control) group and T_i = mean weight of gall bladder (mg) in the drug-treated group.

Treatment

Separate groups of animals received saline (0·25 mL, i.p., control) or morphine (1,2,4 mg kg^{-1}, i.p.), naloxone (2 mg kg^{-1}, i.p.) glibenclamide (0·65 mg kg^{-1}, i.p.) or minoxidil (0·65 mg kg^{-1}, i.p.), 8 min before oral administration

of egg yolk. In the study of the combined treatment, different groups of animals were treated with naloxone (2 mg kg^{-1}, i.p.) glibenclamide (0·65 mg kg^{-1}, i.p.) or minoxidil (0·65 mg kg^{-1}, i.p.) 30 min before morphine treatment.

Statistical analysis

Data are expressed as mean ± s.e. Statistical significance was determined by Student's *t*-test with $P < 0·05$ considered as statistically significant.

Results

The average weight of the gall bladder and percent inhibition of egg yolk-induced gall-bladder emptying after administration of drug or drug combination are shown in Table 1. Administration of morphine dose-dependently inhibited egg yolk-induced gall-bladder emptying; there was a significant increase in average weight of gall bladders of these groups when compared with control. There was a significant decrease in the average weight of gall bladder in the naloxone-treated group, which indicates, naloxone augmented the gall-bladder emptying effect of egg yolk. There was no significant change in the average weight of gall bladder in either the glibenclamide- or the minoxidil-treated groups, suggesting that glibenclamide and minoxidil did not modify the egg yolk-induced gall-bladder emptying. Pretreatment with either naloxone or glibenclamide blocked the inhibitory effect of morphine on egg yolk-induced gall-bladder emptying, showing a significant change in the weight of gall bladders of these groups when compared with the morphine-treated group, whereas minoxidil pretreatment did not modify the inhibitory effect of morphine on egg yolk-induced gall-bladder emptying, with no significant change in the average weight of gall bladder of this group when compared with the morphine-treated group.

Discussion

In the present study, the ATP-dependent K^+ channel-blocker glibenclamide abolished the inhibitory effect of morphine on gall-bladder emptying. Several studies have shown that stimulation of μ- and δ-opioid receptors causes hyperpolarization of brain neurones due to the opening of K^+ channels. Such an action has been suggested to be involved in some effects of morphine-like analgesia and respiratory depression (North 1986; Welch & Dunlow 1993). The ATP-dependent K^+ channel-blocker, glibenclamide, has been reported to antagonize morphine analgesia (Welch & Dunlow 1993; Ocana et al 1993). Further, K^+-channel openers have been reported to produce analgesia after intrathecal administration, suggesting morphine and K^+-channel openers may produce analgesia via an interaction with common second-messenger systems such as calcium (Welch & Dunlow 1993). Similarly, it is reported that ATP-dependent K^+ channels may be involved in the gastroprotective action of morphine (Bhounsule et al 1992). In contrast, Sunita et al (1994) have reported that glibenclamide failed to antagonize the antimotility actions of morphine and suggested the possibility that the intestinal actions of morphine are mediated by a type or sub-type of opioid receptor different from that mediating its analgesic actions.

The presence of egg yolk in the duodenum releases CCK which is a physiological stimulus for the gall bladder and causes contraction of the gall bladder musculature, hence emptying the gall bladder (Byrnes et al 1981; Valsecchi & Toson 1982). In the present study, systemic administration of morphine dose-dependently inhibited egg yolk-induced gall-bladder emptying. Pretreatment with naloxone completely blocked this inhibitory action of morphine. This naloxone-reversible effect was attributed to opioid-induced spasm of the sphincter of Oddi (Paget & Barnes 1964; Valsecchi & Toson 1982) probably through inhibition of acetylcholine release from the cholinergic neurones in the terminal bile ducts (Asaoka & Uouchi 1982). Direct effect of morphine on gall-bladder contraction is unlikely, since acute addition of morphine neither induced contraction of gall-bladder strips nor reduced tonic contractions induced by CCK (Malave & Yim 1992).

Examination of the effect of glibenclamide alone or in combination with morphine on egg yolk-induced gall-bladder emptying provides evidence that the effect of morphine on the biliary tract may be via modulation of the ATP-dependent K^+ channels, as glibenclamide completely blocked the inhibitory effect of morphine on gall-bladder emptying and failed to modify egg yolk-induced gall-bladder emptying. Thus, direct effect of glibenclamide on the gall-bladder and biliary tract is unlikely.

Intrathecal administration of minoxidil produced antinociception which was blocked by glibenclamide and also differentially by opiate antagonists (Welch & Dunlow 1993). In the present study minoxidil neither affected the gall-bladder emptying when given alone nor modified the morphine effect when administered with morphine (1 mg kg^{-1}). It may be possible that the ATP-dependent K^+ channels of the biliary tract may not be sensitive to minoxidil.

The results of the present study and earlier reports (Ocana et al 1990; Welch & Dunlow 1993) suggest that the biliary tract actions of morphine are probably mediated via glibenclamide-sensitive K^+ channels similar to those involved in the analgesic action of morphine.

Table 1. Inhibition of egg yolk-induced gall-bladder emptying in control and drug-treated mice.

Treatment	Dose (mg kg^{-1})	Gall bladder weight (mg) (Mean ± s.e.)	Inhibition of emptying (%)
Saline (i.p. and p.o.)	—	20·35 ± 0·94	—
Saline (i.p. only)	—	7·06 ± 0·47	—
Morphine	1	14·09 ± 0·16[a]	52·89
	2	17·69 ± 0·30[a]	79·98
	4	20·21 ± 0·33[a]	98·94
Naloxone	2	1·57 ± 0·49[a]	—
+ morphine	2	7·54 ± 0·28[b]	3·720
Glibenclamide	0·65	8·53 ± 0·92	—
+ morphine	2	8·26 ± 0·93[b]	9·02
Minoxidil	0·65	7·54 ± 0.16	—
+ morphine	1	14·18 ± 0·56[c]	53·37

n = 9–11. [a] $P < 0·001$ compared with saline,
[b] $P < 0·001$ compared with morphine 2 mg kg^{-1};
[c] $P < 0·5$ compared with morphine 1 mg kg^{-1}.

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