ELSEVIER

European Journal of Pharmacology 287 (1995) 207–209

ejp

Short communication

K^+ channel openers delay intestinal transit and have antidiarrheal activity

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Received 29 September 1995; accepted 10 October 1995

Abstract

The effect of pinacidil and cromakalim, two K_{ATP} channel openers, on intestinal transit and castor oil-induced diarrhea was studied in mice. Both drugs, administered orally, dose dependently inhibited the intestinal propulsion of charcoal, and castor oil-induced diarrhea, comparing favorably with morphine. These results may suggest a new approach for the symptomatic treatment of diarrhea.

Keywords: K_{ATP} channel opener; Pinacidil; Cromakalim; Morphine; Intestinal transit; Diarrhea

1. Introduction

The bowel is one of the major target organs of opioids, and opioids remain the most effective agents for treating diarrhea.

The gastrointestinal effects of opioids are mainly mediated by μ and δ-opioid receptors. Activation of μ and δ-opioid receptors increases the membrane permeability to K^+ (McFadzean, 1988). ATP-sensitive K^+ (K_{ATP}) channels are present in intestinal smooth muscle and epithelial cells (Carl et al., 1992; Du et al., 1994; Franck et al., 1994; Sun and Benishin, 1994; Homaidan and Broutman, 1994), and K^+ channel openers hyperpolarize and relax intestinal smooth cells and stimulate NaCl absorption in villus cells (Sun and Benishin, 1994; Franck et al., 1994; Homaidan and Broutman, 1994).

All together, these data strongly suggest that K_{ATP} channel openers may be of therapeutic value in the treatment of diarrhea. Here we present experimental data that directly support this possibility.

2. Materials and methods

2.1. Animals

Adult male Swiss albino mice (Charles River, Calco, CO, Italy), weighing 24–28 g, were used. They were housed five per cage (26 × 21 × 14 cm) in climatized colony rooms (temperature 21 ± 1°C; humidity 60%) on a natural light-dark cycle and with food and water continuously available. They were acclimatized for one week before the test. All the procedures were carried out according to the EEC ethical regulations for animal research (EEC Council 86/609; D.L. 27/01/1992, No. 116).

2.2. Charcoal intestinal motility

A charcoal suspension (10 ml/kg of a 10% suspension of activated charcoal in 5% methylcellulose) was given orally to 24-h fasted mice (water ad libitum), 45 min after an oral dose of drug or vehicle. The animals were killed by cervical dislocation under ethyl ether anesthesia 15 min after receiving the charcoal and the intestines were carefully removed without stretching and placed lengthwise on moist white filter paper (Mir

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SSDI 0014-2999(95)00658-3

et al., 1978). The length of the intestine (pyloric sphincter to caecum) and the distance traveled by the charcoal as a percentage of that length were evaluated for each animal, and group means were compared and expressed as percentage inhibition:

$$\% \text{ inhibition} = \frac{(\text{mean distance in controls}) - (\text{mean distance in treated})}{\text{mean distance in controls}} \times 100$$

2.3. Castor oil-induced diarrhea

Mice were dosed orally with drug or vehicle immediately before receiving 0.4 ml of castor oil by the same route (Mir et al., 1978).

The animals were individually caged and examined for the presence of diarrhea hourly for 3 h after castor oil challenge. Diarrhea was defined as the presence in the stools of fluid material which stained the absorbent paper placed beneath the cage.

2.4. Drugs and treatments

The K_{ATP} channel openers, cromakalim and pinacidil, were obtained from RBI (Natick, MA, USA) and Sigma Chemical Co. (St. Louis, MO, USA), respectively, and administered orally at the doses of 0.1, 0.5, 1, 5 and 10 mg/kg. All doses were dissolved in dimethyl sulfoxide 0.3 ml and then diluted with distilled water to a final volume of 10 ml. Morphine (morphine sulphate, Salars, Como, Italy) was used as reference antidiarrheal compound and administered orally at the doses of 1, 2 and 5 mg/kg. Control animals received the same volume of vehicle orally. Groups of at least 10 mice per dose were used.

2.5. Statistical analysis

All data are given as means ± S.E.M. and were analyzed for statistical significance by Student's *t*-test and Fisher's exact test when appropriate. D_{50} was calculated according to Tallarida and Jacob (1979).

3. Results

3.1. Effect of K_{ATP} channel openers on intestinal transit

Both pinacidil and cromakalim dose dependently inhibited the intestinal propulsion of charcoal in mice, as shown in Table 1.

The effect was significant starting from the dose of 1 mg/kg in the case of pinacidil, and from the dose of 0.5 mg/kg in the case of cromakalim. The effect was dose dependent in the dose range used, for both pinacidil and cromakalim (52% and 65% inhibition respectively). Under our experimental conditions, the highest dose of morphine (5 mg/kg) produced a 29.2% inhibition of intestinal propulsion.

Table 1
Effect of cromakalim, pinacidil, and morphine on intestinal transit in the mouse

Treatment	Dose mg/kg p.o.	No. of mice	Transit rate inhibition (%)
Cromakalim	0.1	10	27.99 ± 1.9
	0.5	10	45.45 ± 2.1 [a]
	1	10	49.10 ± 2.3 [a]
	5	10	57.26 ± 1.9 [a]
	10	10	65.50 ± 2.5 [a]
	D_{50} (mg/kg)		1(0.3–3.2)
Pinacidil	1	20	10.30 ± 0.6 [a]
	5	15	30.25 ± 1.2 [a]
	10	15	52.13 ± 1.8 [a]
	D_{50} (mg/kg)		9(13.4–6.2)
Morphine	1	25	16.26 ± 0.7
	5	8	29.20 ± 1.1

Compounds were administered 45 min before charcoal suspension. Each value represents the mean ± S.E.M. [a] Significant difference (Student's *t*-test) from the respective control group. $P < 0.05$, at least.

3.2. Effect of K_{ATP} channel openers on castor oil-induced diarrhea

As shown in Table 2, under our experimental conditions mice treated either with pinacidil or with cromakalim were protected from castor oil-induced diarrhea, over the 3-h observation period. The degree of protection was dose related. In mice given 5 and 10 mg/kg of pinacidil, diarrhea was observed only 3 h after castor oil administration, in 8 and 4 out of 20 animals, respectively; in mice given 0.5, 1 and 5 mg/kg

Table 2
Effect of cromakalim, pinacidil, and morphine on castor oil-induced diarrhea in the mouse

Treatment	Dose mg/kg p.o.	No. of mice	% of mice protected after castor oil		
			1 h	2 h	3 h
Vehicle	–	25	85.7	42.8	8.5
Cromakalim	0.1	10	70	40	10
	0.5	16	100	87.5	50
	1	16	100	87.5	62.5 [a]
	5	16	100	87.5	75 [a]
Pinacidil	1	25	92	60	20
	5	20	100	100	60 [a]
	10	20	100	100	80 [a]
Morphine	2	21	86	34	20
	5	10	90	70	50

Compounds were administered immediately before castor oil (0.4 ml p.o.). [a] Significant difference (Fisher's test) from the control group. $P < 0.05$, at least.

of cromakalim, diarrhea was observed in 2 out of 16 animals 2 h after castor oil administration and in 8, 6 and 4 out of 16 animals, respectively, 3 h after castor oil.

In contrast, 57.2% and 91.5% of mice treated with saline had diarrhea at the 2nd and 3rd hour, respectively, while 30% and 50% of mice treated with morphine at the dose of 5 mg/kg per os had diarrhea at the same times after castor oil administration.

4. Discussion

Our present data show that in mice pinacidil and cromakalim, two drugs that open ATP-sensitive K^+ (K_{ATP}) channels, delay intestinal transit and protect against castor oil-induced diarrhea.

The physiological role of K_{ATP} channels in intestinal smooth muscle is unclear. There is no evidence that these channels are involved in the inhibitory effect of non-adrenergic non-cholinergic neurotransmitters, or that they have a physiological role in capsaicin- or calcitonin gene-related peptide-induced responses in the small intestine (Franck et al., 1994).

Our present data may suggest that they play an important role in the mechanism of action of endogenous opioids at the gastrointestinal level. Indeed, pro-opiomelanocortin as well as pro-enkephalin and pro-dynorphin are synthesized and processed locally in gut tissue, and μ- and δ-opioid receptors are found in the gastrointestinal wall (O'Donohue and Dorsa, 1982).

If our results are confirmed in other animal species and in humans, a new approach to the symptomatic treatment of diarrhea could be suggested. In this respect, it is perhaps worth mentioning that K_{ATP} openers, which are potent antihypertensive drugs and markedly reduce systolic and diastolic blood pressure in hypertensive subjects, have practically no effect on the blood pressure of normotensive subjects (Singer et al., 1989). Hyperglycemia is only seen with diazoxide, but not with other K_{ATP} channel openers (Garrino et al., 1989).

References

Carl, A., S. Bowen, C.H. Gelband, K.M. Sanders and J.R. Hume, 1992, Cromakalim and lemakalim activate Ca^{2+} dependent K^+ channels in canine colon, Pflüg. Arch. 421, 67.

Du, C., A. Carl, T.K. Smith, K.M. Sanders and K.D. Keef, 1994, Mechanism of cyclic AMP-induced hyperpolarization in canine colon, J. Pharmacol. Exp. Ther. 268, 208.

Franck, H., A. Puschman, V. Schusdziarra and H.D. Allescher, 1994, Functional evidence for a glibenclamide sensitive K^+ channel in rat ileal smooth muscle, Eur. J. Pharmacol. 271, 379.

Garrino, M.G., T.D. Plant and J.C. Henquin, 1989, Effects of putative activator in K^+-channels in mouse pancreatic β-cells, Br. J. Pharmacol. 98, 957.

Homaidan, F.R. and G. Broutman, 1994, Regulation of ileal electrolyte transport by K^+ channel activators, Eur. J. Pharmacol. 271, 561.

McFadzean, I., 1988, The ionic mechanism underlying opioid action, Neuropeptides 11, 173.

Mir, G.N., R.L. Alioto, J.W. Sperow, J.R. Eash, J.B. Krebs and J. Yelnosky, 1978, In vivo antimotility and antidiarrheal activity of Lidamidina Hydrochloridre (WHR-1142A), a novel antiarrheal agent, Arzneim.-Forsch./Drug Res. 28 (II), 1448.

O'Donohue, T.L. and D.M. Dorsa, 1982, The opiomelanotropinergic neuronal and endocrine systems, Peptides 3, 353.

Singer, D.R.J., N.D. Markandu, M.A. Miller, A.L. Sugden and G.A. MacGregor, 1989, Potassium channel stimulation in normal subject and in patients with essential hypertension: an acute study with cromakalim (BRL 34915), J. Hypertens. 7, 294.

Sun, Y.D. and C. Benishin, 1994, K^+ channel openers relax longitudinal muscle of guinea pig ileum, Eur. J. Pharmacol. 271, 453.

Tallarida, R.J. and L.S. Jacob, 1979, The Dose-Response Relation in Pharmacology (Springer-Verlag, New York).