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Characteristics of the premature phase 3 of the migrating motor complex evoked by pirenzepine administration in the small bowel of non-fasted sheep

Krzysztof W. Romański

Department of Biostructure and Animal Physiology, Faculty of Veterinary Medicine, University of Environmental and Life Sciences, Wrocław, Poland

Abstract: In sheep, pirenzepine (Pi) blocks muscarinic receptors thus inhibiting the gastrointestinal motility. However, its small doses also evoke the premature phase 3 of the migrating motor complex (MMC). The aim of this study was to specify further the character of stimulatory alterations caused by the drug in non-fasted sheep. The experiments were carried out in four conscious rams with sewn duodenal strain gauge force transducer and duodeno-jejunal bipolar electrodes. Following the control period, 0.15 M NaCl or Pi, at the dose 0.02 mg/kg body weight, were slowly administered into the jugular vein and motor-myoelectric activity recordings were continued until normal motility returned. In the course of most experiments Pi evoked the premature phase 3 preceded by 3-5 min inhibition in non-fasted rams. Its duration in the duodenum was shorter than in the jejunum and the same difference was observed in normal phase 3 of the MMC. Amplitude and duration of the spike bursts forming the premature phase 3 was not significantly different from normal phase 3 of the MMC cycle. The premature phase 3 exhibited a regularly propagating character in most cases, but occurred also in incomplete or disorganized forms. Usually, its character thus closely resembled the normal phase 3 of the MMC. It appears that Pi, at the low doses, triggers the premature phase 3 via the blockade of neuronal M_1 receptor subtype that provokes the secondary activation of acetylcholine release. Induction of premature phase 3 by a small dose of Pi resembles the effect observed in monogastric species.

Key words: Sheep, small bowel, pirenzepine, premature phase 3, migrating motor complex

INTRODUCTION

In the fasting state, gastrointestinal motility attains the form of a repeatable pattern termed the migrating motor or myoelectric complex (MMC) [1]. This is most frequently composed of four phases. During phase 1 of the MMC rather no contractions are present, but the subseuqent phase 2 represents a gradually increasing incidence of irregular contractions or spike bursts. Phase 3 of the MMC can be characterized by intense, regular, and propagated contractions. When the myoelectric activity is recorded, the maximal spike bursts forming this phase are superimposed on every slow wave. Phase 4 of the MMC comprises irregular contractions, similar to those during phase 2, but is not always observed. Phase 3 of the MMC, as the most propulsive activity during the cycle, is responsible first of all for stirring and transporting residual chyme along the digestive tube. It can also arrive in the different regions with or sometimes without a clear connection with the whole cycle as the premature phase 3 [3]. The MMC pattern strictly undergoes the neural control where the cholinergic system plays a principal role [3, 4]. Administration of drugs acting *via* cholinergic receptors evokes substantial alterations in the gastrointestinal motility, including also the MMC pattern [5, 6]. The action of these drugs engages the nicotinic and muscarinic cholinergic receptors. Atropine is the most common drug used for this purpose, but belongs to an unspecific muscarinic inhibitors. Pirenzepine (Pi) has been primarily recognized as the drug distinguishing between the different subclasses of muscarinic receptors [7]. Its affinity to the M_1 cholinergic receptors is greater than to the other muscarinic receptor subtypes [8, 9]. In monogastris, Pi administered in larger doses inhibits, as the primary responses, the interdigestive motility although the biphasic response was also denoted. When applied in a small dose it induces the premature phase 3 of the MMC [10-12]. In sheep, besides the various alterations in the gastrointestinal motility [13-22], Pi also evokes the premature phase 3 mostly when applied in a low dose [23]. These results suggest that the inhibitory effect of Pi exhibits a dose-response character. There is no precise data regarding the character of premature phase 3 of the MMC cycle in sheep. Therefore, the aim of this study was to describe further the premature phase 3 of the MMC evoked by Pi in the ovine small bowel.

MATERIALS AND METHODS

Animal preparation. The experiments were performed on 4 adult healthy Polish Merino-breed rams each weighing 38-41 kg. After 24-h fast, the animals underwent general and local anesthesia and surgical implantation of five serosal bipolar platinum electrodes, embedded in a Teflon coat, onto the duodenal bulb, 6 cm below the pyloric ring, the duodenum,

Corresponding author: Prof. Krzysztof W. Romański, Department of Biostructure and Animal Physiology, Faculty of Veterinary Medicine, University of Environmental and Life Sciences, Norwida 31, 50-375 Wrocław, Poland.
E-mail: krzysztof.romanski@up.wroc.pl

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50 cm from bulbar electrode, two electrodes onto the jejunum, 200 and 300 cm from duodenal electrode, and one ileal electrode, 110 cm before the caecum. Additionally, one strain gauge force transducer (RB Products, Madison), calibrated before implantation, was sewn just nearby the duodenal electrode. Marked electrode wires were exteriorized over the skin, fixed to the skin at the right side of the abdomen and, in the course of each experiment, were connected with the recording apparatus (electroencephalograph Reega Duplex TR XVI, Alvar Electronic, Paris, also adapted for mechanical recordings).

Experimental design. At least 8-10 days were allowed for postsurgical recovery. The experiments were performed in non-fasted animals. Animals were habituated for the experiments. Drinking water was not limited except in the course of the experiment. The rams were deprived of food overnight before the myoelectric and motor recordings. Before each experiment the jugular vein was catheterized with a thin polyethylene tube. During control experiments, 5 ml of sterile 0.15 M NaCl was given intravenously through the indwelling intravenous cathether over 30 s in the course of phase 2b of the second MMC, identified in the duodenum. During the remaining experiments, after recording 2 consecutive MMCs, pirenzepine dihydrochloride (Pi, Sigma, St. Louis) was introduced through the intravenous cathether at the dose of 0.02 mg/kg of body weight during the duodenal phase 2b of the MMC. All the experiments were repeated on separate days on the same sheep. Following the experiments the animals underwent euthanasia and the positions of the electrodes and strain gauge force transducer were further controlled. Other details of the experimental design have been described elsewhere [24].

Data elaboration. The MMC cycle and its phases were identified in the duodenoum and jejunum according to the criteria described by Code and Marlett [1] with some improvements [24]. The MMC cycles before and after Pi administration were analysed by visual inspection. The detailed character of normal and premature phases 3, their duration, and time lag from the onset of Pi administration was determined. The amplitude and duration of the spike bursts creating the normal and premature phases 3 of the MMC were also measured. Statistical significances were calculated using the Student's *t*-test for paired values, preceded by one-way analysis of variance according to Snedecor and Cochran [25]. Statistical significances were labelled when $p<0.05$ or less. Consequently, 3 steps of statistical significances were distinguished: $p<0.05$, $p<0.01$, and $p<0.001$.

RESULTS

The recording of duodenal motor activity by means of the strain gauge force transducers closely resembled the myoelectric activity registered from the adjacent electrode (Fig. 1). Slow injection of 0.15 M NaCl caused no effect on small-intestinal myoelectric activity. Administration of Pi at a small dose induced the premature phase 3 of the MMC. These stimulatory changes arrived following the inhibitory period lasting some minutes, and usually were longer in the jejunum than in the duodenum (Table 1).

Tables 2-4 characterize more precisely the premature phase 3 of the MMC evoked by Pi administration. In non-fasted animals the premature phase 3 usually, but not always, migrated through these 3 recording channels (electrodes 2-4). Durations of the premature phase 3 of the MMC were longer in the duodenum than in the jejunum, but shorter than the duration of normal phase 3 of the MMC (Table 2).

Table 1 Time lag between pirenzepine (Pi) administration and the arrival of premature phase 3 of the MMC in non-fasted sheep.

		Duodenum	Jejunum 1	Jejunum 2
Pi 0.02 mg/kg	n =	5	8	7
	mean	266.3	387.2	511.0
	±S.D.	48.9	86.4	134.6

Explanations: values in seconds. Duodenum, jejunum 1, jejunum 2 – electrode positions.
Other explanations as in Material and Methods

Table 2 Duration of normal (control) and premature phase 3 of the MMC evoked by Pi administration in non-fasted sheep.

		Duodenum	Jejunum 1	Jejunum 2
Normal phase 3	n =	8	8	7
	mean	248.6	304.9	378.5
	±S.D.	52.3	71.7	95.6
Premature phase 3	n =	5	8	7
	mean	112.2**	195.3	284.9
	±S.D.	31.0	70.8	92.4

Explanations: statistical significance versus relevant control value – **$p<0.01$. Other explanations as in Table 1.

No premature phase 3 nor any excitatory changes were observed in the duodenal bulb and ileum in response to Pi administration (Fig. 1).

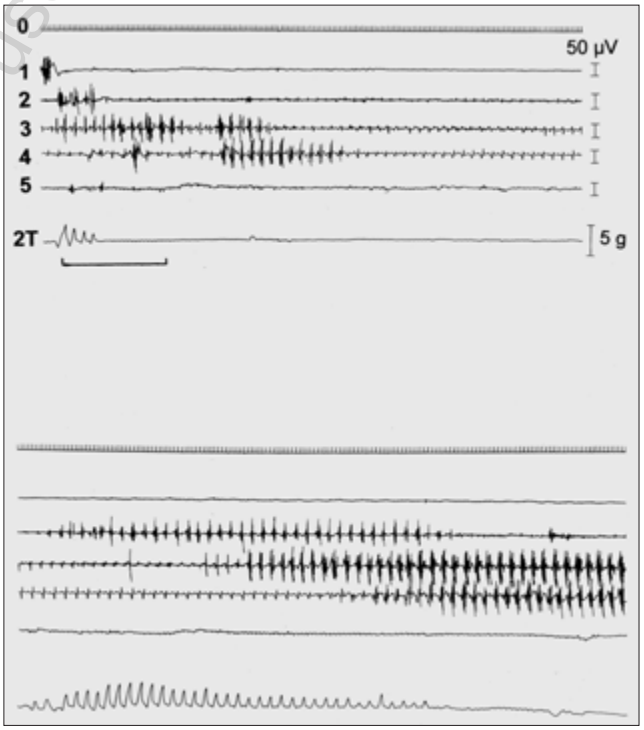

Figure 1 Primary (inhibitory) and secondary (stimulatory, the premature phase 3) effects of pirenzepine (Pi) administration at the small dose during phase 2b of the MMC in non-fasted sheep.
Explanations: 0 – time in seconds. Electrode positions: 1 – duodenal bulb, 2 – duodenum, 3 – jejunum 1, 4 – jejunum 2, 5 – ileum. 2T – strain gauge force transducer attached near the electrode 2. Low bar: Pi administration at the dose 0.02 mg/kg i.v. 50 µV - electrode calibration. 5 g – calibration of strain gauge force transducer.

The premature phase 3 of the MMC evoked by Pi was propagating in about 63% of the phases observed here (Table 3). Their character was different (as shown in Table 3), but the most typical was the pattern migrating from the duodenum to the jejunum, i.e. from channel 2 to 4 (Fig. 1).

Table 3 Character of normal (control) and premature phase 3 of the MMC evoked by Pi administration in non-fasted sheep.

	Normal phase 3 (% of total)	Premature phase 3 (% of total)
Not ectopic	75	0
duodenum	25	38
Ectopic		
jejunum	0	25
Abortive	0	50
Disorganized (irregular)	0	25
Propagated	100	63
Retropropagated	0	0
Non-propagated (stationary)	0	0
Isolated	0	13
Fragmentary	0	13
Doubled	0	13
Lack of phase 3	0	0

Explanations: n (total number) = 8. Other explanations as in Table 1.

Amplitude of the spike bursts of premature phase 3 was significantly higher in the jejunum as compared with the duodenum (Table 4). In the latter, the amplitude of the spike bursts forming the normal phase 3 of the MMC was slightly but not significantly higher than that of the premature phase 3 (Table 4).

Table 4 Average amplitude of the spike bursts forming normal (control) and premature phase 3 of the MMC evoked by Pi administration in non-fasted sheep.

		Duodenum	Jejunum 1	Jejunum 2
	n =	5	8	7
Normal phase 3	mean	79.8	118.9***	96.5
	±S.D.	26.3	28.6	32.7
	n =	8	8	7
Premature phase 3	mean	51.6	115.3***	86.4*
	±S.D.	12.4	21.6	23.9

Explanations: values in μV. Statistical significances *vs* relevant duodenal value - *p<0.05;
***p<0.001. Other explanations as in Table 1.

Duration of the spike bursts of premature phase 3 was significantly longer in the jejunum than in the duodenum, and was very similar to that of the spike bursts forming normal phase 3 of the MMC (Table 5).

Table 5 Duration of the spike bursts of normal (control) and premature phase 3 of the MMC evoked by Pi administration in non-fasted sheep.

		Duodenum	Jejunum 1	Jejunum 2
	n =	5	8	7
Normal phase 3	mean	1.19	1.88*	1.66
	±S.D.	0.16	0.25	0.31
	n =	8	8	7
Premature phase 3	mean	1.06	1.91*	1.74
	±S.D.	0.09	0.19	0.27

Explanations: values in seconds, statistical significances versus relevant duodenal value –
*p<0.05. Other explanations as in Table 1.

DISCUSSION

Pi, when given in the small dose, transiently inhibits the spike bursts and then evokes the migratory premature phase 3 of the MMC in man and monogastric animals [11, 26]. Similar effects have been reported following the administration of telenzepine that induced the premature phase 3 of the MMC - for example, in canine small bowel [27]. However, in man and in sheep no such effect was observed in response to this drug [19, 28]. Telenzepine is principally the M_1 cholinergic receptor antagonist [29] while Pi may act through either M_1 or M_2 cholinergic receptors [30, 31]. Its action *via* other muscarinic receptor subtypes is not excluded, however, but it appears that the affinity of Pi to these subtypes of muscarinic receptors is much lower [8]. The roles of M_4 and M_5 receptor subtypes in the control of gastrointestinal motility have not been precisely defined [32, 33]. The role of M_2 receptor subtypes in gastrointestinal motility is also not well known. When postsynaptic action is considered, the role of M_2 receptor subtype can interfere with the stimulation of the M_3 receptor subtype and in presynaptic action, the effect of stimulation of the M_2 and M_1 receptor subtypes can be common. Indeed, the inhibitory functions of M_1 (located presynaptically on enteric neurons and on endocrine cells, see below) and stimulatory functions of M_3 (located on the smooth muscles) cholinergic receptor subtypes have been the most clearly recognized in the control of gastrointestinal motility [34-39]. In the present study, the smallest dose of Pi evoked the stimulatory response in sheep, as also observed previously [23]. Although the inhibitory M_2 receptor subtype can also be located presynaptically [40, 41], its affinity to Pi is smaller than that of M_1 receptor subtype [8, 9]. Thus, it can be presumed that induction of premature phase 3 of the MMC by the smallest dose of Pi, as observed also in sheep [23], engaged mostly the M_1 receptor subtype. Its action might be expected to be rather peripheral since the ability of the drug to cross the blood-brain barrier is limited [42]. Therefore, it can be assumed that the induction of premature phase 3 of the MMC can occur thanks to the activation of an intrinsic cholinergic pathway [43], although the detailed mechanism of this action remains to be elucidated. It was found recently that the activation of nicotinic receptors might be linked with the modulation of muscarinic receptors [44]. Since not only postsynaptic, but also presynaptic nicotinic receptors are involved in the gastrointestinal motility, the interaction at the presynaptic level between M_1 and nicotinic receptors may enhance the stimulatory action of Pi [45], but this does not appear to be the case in the induction of the premature phase 3. It has been reported that rapid injection of 1 mmol HCl also triggers the premature phase 3 of the MMC in ovine duodenum, and vagotomy abolishes this effect [46]. It can be inferred from this study that endocrine mechanisms could also participate in this response and an imprecise vagal component also contributes to it. Whether this comprises the mechanisms inducing the premature phase 3 or not is not known. Blockade of M_1 receptor subtype may intensify the small-intestinal peristalsis, and a similar effect is evoked by motilin [12, 47]. Since M_1 receptors are present on motilin cells such an action is possible also in sheep in which the role of motilin in the control of the MMC is limited [48, 49]. The most probable mechasnisms inducing the premature phase 3 by Pi are depicted in Fig. 2.

Finally, it is concluded that in sheep a small dose of Pi evokes the premature phase 3 of the MMC, and sometimes

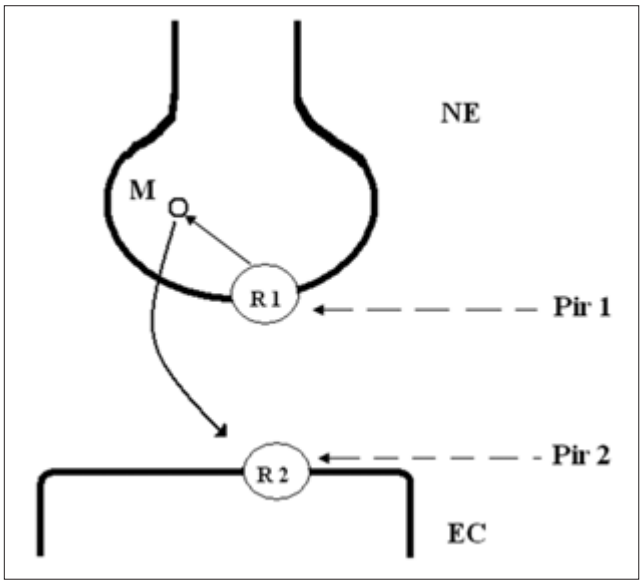

Figure 2 Suggested neural mechanism of pirenzepine (Pi) action on intestinal motility in sheep. In the small dose of Pi (Pir 1) it inhibits the most probably M_1 cholinergic receptor subtype and exhibits the greatest affinity to this receptor. Inhibition of autoreceptor directly or indirectly stimulates the processes related to the release of mediator to the synaptic cleft and inducing the stimulatory response, i.e. the premature phase 3 of the MMC. This effect is relatively short since the action of the small dose of Pi is not so long. In the higher dose of Pi (Pir 2) its action is similar to that of the small dose but at the beginning the inhibitory action predominates and can be longer. After some time, the stimulatory effect prevails and inhibition of motor activity is replaced by secondary stimulatory response.
Explanations: NE – nerve ending, EC – effector (smooth muscle) cell, M - synaptic vesicle with neuromediator, R_1 – presynaptic (most probably M_1) cholinergic receptor subtype, R_2 – postsynaptic (most probably M_3) cholinergic receptor subtype.

the nonmigrating activity. It appears that the small dose of Pi acted mostly *via* M_1 receptors, as the most sensitive to Pi. In sheep, the effect of Pi on small-intestinal motility is thus roughly similar to that in monogastrics, although in non-fasted sheep the character of premature phase 3 of the MMC is not uniform. These results can be useful in the further studies on Pi as a drug used in gastroenterology.

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