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Abstract

The onset of beta-methyl-digoxin action was investigated by the potentiation of the adenosine response in guinea pigs and rats, and compared with that of digoxin and dipyridamole. A number of i.v. infusions of adenosine were given to determine the mean control adenosine response and its 95% confidence limits. After oral administration of the drugs, successive infusions of adenosine were continued until a drug-induced potentiation of the adenosine response was observed. The time of appearance of the potentiated adenosine response was marked as the onset of action of the drugs. The onset of action in guinea pigs was 9 to 12 min for 0.2 to 0.4 mg/kg of beta-methyl-digoxin, 90 to 100 min for 0.2 mg/kg of digoxin and 25 min for 5 mg/kg of dipyridamole. The maximal potentiation was 48.8 to 53.8% at 18 to 21 min for beta-methyl-digoxin, 74.5% at 130 min for digoxin and 74.8% at 80 min for dipyridamole. Adenosine infused i.v. into rats produced heart block, as in guinea pigs. However, in rats, the adenosine response was not potentiated by beta-methyl-digoxin and digoxin. Dipyridamole at a dose as high as 200 mg/kg produced 25.8% potentiation at 36 min after oral administration to rats.

KEYWORDS: β -methyl-digoxin, digoxin, dipyridamole, onset of action, guinea pigs and rats

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DETERMINATION OF THE ONSET OF β -METHYL-DIGOXIN ACTION BY POTENTIATION OF THE ADENOSINE RESPONSE IN GUINEA PIGS

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Abstract. The onset of β -methyl-digoxin action was investigated by the potentiation of the adenosine response in guinea pigs and rats, and compared with that of digoxin and dipyridamole. A number of i.v. infusions of adenosine were given to determine the mean control adenosine response and its 95 % confidence limits. After oral administration of the drugs, successive infusions of adenosine were continued until a drug-induced potentiation of the adenosine response was observed. The time of appearance of the potentiated adenosine response was marked as the onset of action of the drugs. The onset of action in guinea pigs was 9 to 12 min for 0.2 to 0.4 mg/kg of β -methyl-digoxin, 90 to 100 min for 0.2 mg/kg of digoxin and 25 min for 5 mg/kg of dipyridamole. The maximal potentiation was 48.8 to 53.8 % at 18 to 21 min for β -methyl-digoxin, 74.5 % at 130 min for digoxin and 74.8 % at 80 min for dipyridamole. Adenosine infused i.v. into rats produced heart block, as in guinea pigs. However, in rats, the adenosine response was not potentiated by β -methyl-digoxin and digoxin. Dipyridamole at a dose as high as 200 mg/kg produced 25.8 % potentiation at 36 min after oral administration to rats.

Key words : β -methyl-digoxin, digoxin, dipyridamole, onset of action, guinea pigs and rats.

Adenosine produces a transient period of heart block associated with prolongation of the P-R interval and bradycardia in guinea pigs and rats (1). It is known that the response to an intra-atrial injection of adenosine is potentiated by cardiac glycosides injected intravenously (i.v.) in guinea pigs (2, 3). Using the method of the potentiation of the adenosine response, the duration of action and the elimination of cardiac glycosides were demonstrated in guinea pigs (2, 4). β -Methyl-digoxin was developed with the aim of improving the absorption of digoxin, and it has been reported that β -methyl-digoxin is absorbed more rapidly than digoxin (5, 6). However, the speed of onset of the action of β -methyl-digoxin, administered orally similarly to the clinical situation, has not been determined in animals. Since it was found in this study that an i.v. injection of adenosine also produced definite heart block in guinea pigs and rats, the potentiation of the adenosine response was applied to the determination of the onset of β -methyl-digoxin action. The results were compared with those obtained with digoxin and

dipyridamole which also potentiate the adenosine response (7).

MATERIALS AND METHODS

Reagents. β -Methyl-digoxin (Boehringer-Mannheim, West Germany), kindly donated by Yamanouchi Pharmaceutical Co., Ltd. (Tokyo, Japan), digoxin (JP X, Yamanouchi) and dipyridamole (Yamanouchi) were suspended in 0.5 % carboxymethylcellulose sodium (CMC) solution at a volume of 2 ml/kg body weight.

Potentiation of Adenosine Response. The investigations were carried out on male guinea pigs weighing 215 to 400 g and male Wistar rats weighing 250 to 300 g. Food and water were available *ad libitum*. Anesthesia was induced with urethane (i.p.) at 1.7 g/kg and 1.0 g/kg, in guinea pigs and rats, respectively. A cannula was inserted into the left jugular vein for i.v. infusion of adenosine. Adenosine (Nakarai Chemicals, Ltd., Kyoto, Japan) dissolved in 0.9 % NaCl solution at a concentration of 0.4 mg/ml was infused for 4 sec with an infusion speed of 2.9 ml/min using a Harvard Apparatus Compact Infusion Pump (Harvard, Type 975, U.S.A.). The adenosine-induced heart block (adenosine response) was monitored on an EEG recorder (lead II). Adenosine was infused 6 to 10 times at intervals of 3 or 5 min to determine the mean control adenosine response and its 95 % confidence limits. The test drugs were then administered orally (p.o.) by a gastric tube, and the regular infusion of adenosine was continued until the adenosine response was significantly potentiated by the drug. A group of 6 animals was used for each dose of the drugs. Results were evaluated using Student's *t* test. The experiments were carried out at $22 \pm 2^\circ\text{C}$.

Influence of Urethane-Anesthesia on the Passage of Charcoal Meal. Guinea pigs and rats were fasted for 17 h before the experiment. Each animal anesthetized with urethane was administered p.o. 4 ml/kg of charcoal meal (5 % charcoal in 10 % CMC solution). Arbitrary times after the administration of the charcoal meal, groups of 5 animals were sacrificed, the small intestine removed, and the passage of charcoal in the small intestine measured and expressed as a percentage (%) of the total length of the small intestine.

RESULTS

Constancy of adenosine response. The adenosine-induced heart block (adenosine response) was found from 5.7 to 9.2 sec, when adenosine (193 to 352 $\mu\text{g}/\text{kg}$) was infused i.v. for 4 sec with the infusion pump at intervals of 3 min in guinea pigs. The response remained at a constant level for 2 to 3 h. An i.v. infusion of adenosine in rats also produced heart block, and this response remained constant like the response in guinea pigs.

The responses remained constant within the 95 % confidence limits of the mean of the initial 10 responses. A 0.5 % CMC solution administered p.o. had no effect on the response to successive infusion of adenosine in guinea pigs and rats.

Potentiation of adenosine response in Guinea pigs. The effect of β -methyl-digoxin (0.2 mg/kg) administered p.o. on the adenosine response in guinea pigs is shown in Fig. 1. The control adenosine response was 7.21 ± 0.10 (S.D.) sec. Following the administration of β -methyl-digoxin, the adenosine response was markedly potentiated from 7.21 to 8.00 sec at 9 min. A potentiation ranging from 9.7 to 11.0 sec was observed from 12 to 42 min after the drug administration. The mean

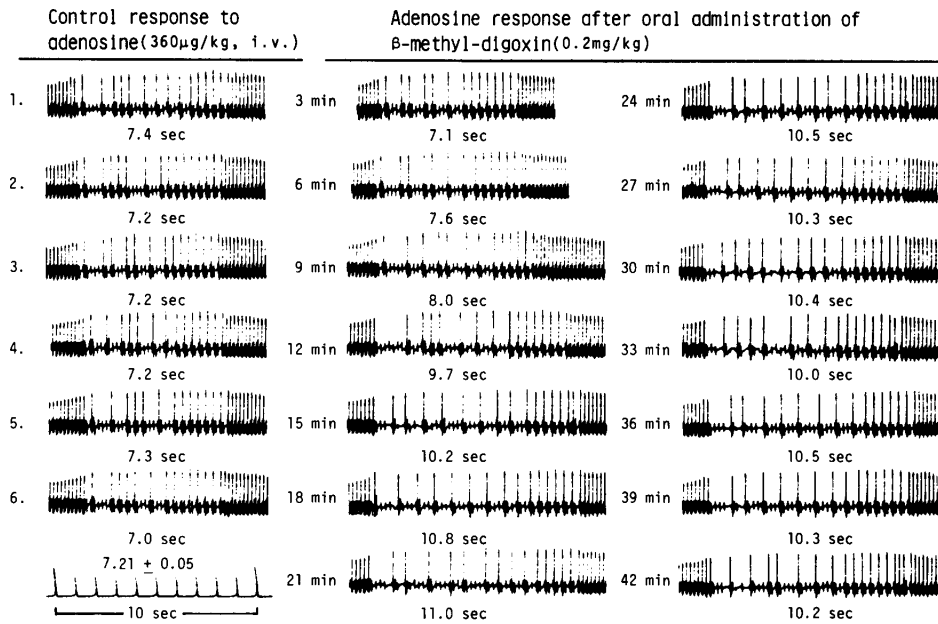


Fig. 1. Effect of β -methyl-digoxin administered p.o. on the adenosine response in the guinea pig. The left panel shows the control responses to adenosine given at 3-min intervals. The other panels show the responses to adenosine administered i.v. at 3-min intervals after an oral administration of the drug.

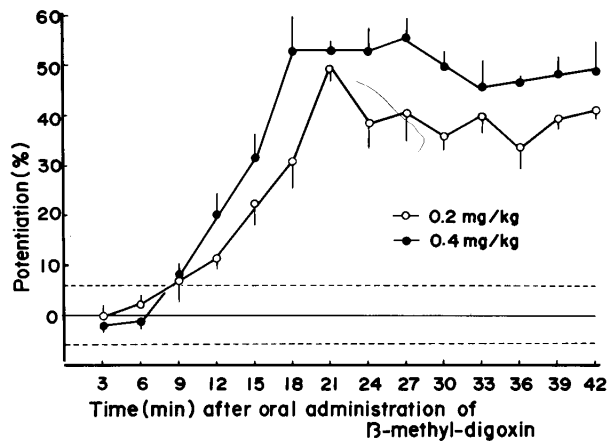


Fig. 2. Potentiating of β -methyl-digoxin (0.2 and 0.4 mg/kg, p.o.) on the adenosine response in guinea pigs. The solid horizontal line shows the mean control response to adenosine and the broken lines show its 95% confidence limits.

potentiation (%) of the adenosine response by β -methyl-digoxin (0.2 and 0.4 mg/kg) is shown in Fig. 2. Following the administration of the drug, the adenosine response was significantly potentiated: $11.1 \pm 1.8\%$ ($p < 0.001$) at 12 min for 0.2 mg/kg and $8.3 \pm 1.7\%$ ($p < 0.01$) at 9 min for 0.4 mg/kg. The maximal effects of this glycoside were $48.8 \pm 2.8\%$ potentiation at 21 min for 0.2 mg/kg, and $53.8 \pm 7.7\%$ potentiation at 18 min for 0.4 mg/kg.

The mean potentiation by digoxin (0.2 mg/kg) and dipyridamole (5 mg/kg) are shown in Fig. 3. After the administration of the drugs, the first potentiations were $40.3 \pm 2.2\%$ ($p < 0.001$) at 100 min for digoxin and $10.0 \pm 4.3\%$ ($p < 0.01$) at 25 min for dipyridamole. The maximal effect by dipyridamole was $74.8 \pm 8.9\%$ at 80 min after the administration of the drug. For the maximal effect by digoxin more than 130 min were required in this experiment. However, the potentiation by digoxin at 130 min was $74.5 \pm 9.0\%$, and was higher than the maximal effect of β -methyl-digoxin (0.2 mg/kg). β -Methyl-digoxin and digoxin at 0.1 mg/kg and dipyridamole at 1 mg/kg did not significantly potentiate the adenosine response in guinea pigs.

The time of onset of action and the maximal effect of the drugs are shown in Table 1.

Potentiation of adenosine response in rats. The adenosine response was not increased by β -methyl-digoxin and digoxin in dose up to 3 mg/kg. Dipyridamole at a dose of 200 mg/kg produced the first potentiation ($10.6 \pm 3.2\%$, $p < 0.05$) 18 min and the maximal effect ($25.8 \pm 2.6\%$) 40 min after the oral administration.

Influence of urethane anesthesia on the passage of charcoal meal. In order to observe the influence of urethane anesthesia on the transport of the contents of the stomach, the passage of charcoal meal in the small intestine of animals was examined.

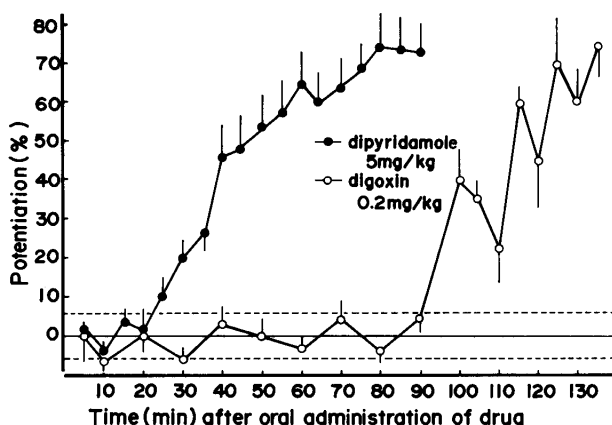


Fig. 3. Potentiating effect of digoxin (0.2 mg/kg, p.o.) and dipyridamole (5 mg/kg, p.o.) on the adenosine response in guinea pigs. The solid horizontal line shows the mean control response to adenosine and the broken lines show its 95% confidence limits.

TABLE 1. ONSET OF ACTION AND TIME OF MAXIMAL EFFECT OF β -METHYL-DIGOXIN, DIGOXIN AND DIPYRIDAMOLE AS DETERMINED BY POTENTIATION OF THE ADENOSINE RESPONSE IN GUINEA PIGS.

	β -Methyl-Digoxin 0.2-0.4 mg/kg, p.o.	Digoxin 0.2 mg/kg, p.o.	Dipyridamole 5 mg/kg, p.o.
Onset of action	9-12 min	90-100 min	25 min
Maximal effect	18-21 min	> 130 min	80 min

TABLE 2. INHIBITORY EFFECT OF URETHANE ANESTHESIA ON THE PASSAGE OF CHARCOAL MEAL IN THE SMALL INTESTINE OF ANIMALS

Time after administration of charcoal meal	Passage of charcoal (%)	
	Urethane-anesthetized	Un-anesthetized
Guinea-pigs		
5 min	9.1 \pm 1.2	
10 min	17.3 \pm 2.2	54.8 \pm 6.4
Rats		
30 min	0	70.7 \pm 9.1
40 min	5.5 \pm 1.8	

Each value represents the mean \pm S.D. of five animals.

As shown in Table 2, the passage of charcoal in anesthetized guinea pigs was 9.1 and 17.3 % exactly 5 and 10 min after the administration of charcoal meal, respectively, while the passage of charcoal in unanesthetized guinea pigs was 54.8 % at 10 min. On the other hand, the passage of charcoal into the small intestine in anesthetized rats was not observed until 30 min, and only slight passage (5.5 %) was found 40 min after the administration of charcoal meal. Although the passage of charcoal in guinea pigs and rats was markedly inhibited by urethane anesthesia, it was shown that charcoal in anesthetized guinea pigs reaches the small intestine shortly after the administration of charcoal meal.

DISCUSSION

Many previous workers have stated that cardiac glycosides and dipyridamole injected i.v. potentiate the pharmacological actions of adenosine (2-4, 7, 8). Since the adenosine response were obtained by intra-atrial injections of adenosine in their investigations, it was necessary to use complicated procedures such as giving artificial respiration to the animals after open-chest surgery and tying cannula to the tip of the atrium. This investigation was carried out in order to determine whether successive i.v. infusions adenosine could be used to detect the onset of action of cardiac glycosides by means of the potentiation of the adenosine response.

In the present study, it was demonstrated that the response to successive i.v.

infusions of adenosine, given at intervals of 3 min, remained constant for 2 to 3 h in guinea pigs and rats. It was also shown that this method can be applied to the determination of the onset of action of drugs even when administered p.o. in order to test the effect under conditions similar to the clinical situation. These experiments were carried out in animals under urethane anesthesia. It has been reported that the transport of the content of the stomach is impaired by anesthesia of this kind. In order to observe the influence of urethane anesthesia on the transport of the contents out of the stomach, the passage of charcoal meal in the small intestine of animals was examined. It was shown that charcoal in anesthetized guinea pigs reached the small intestine soon after the administration of charcoal meal, while the passage of charcoal in anesthetized rats was not observed until 30 min after the administration (Table 2). The potentiation of the adenosine response by drugs was more dramatic in guinea pigs than in rats, as observed by Stafford (7).

Abendroth and Neudert (9), who used the method of intra-cardial pressure measurements, estimated that the onset of action of β -methyl-digoxin in patients is about 8 min after oral administration. Our results based on the potentiation of the adenosine response in guinea pigs are very similar to their values. The findings obtained with both methods confirm the rapid onset of action of β -methyl-digoxin even when administered p.o.

0.2 mg/kg (p.o.) of digoxin potentiated significantly the adenosine response in guinea pigs (Fig. 3), and the time of onset of its action was 90 to 100 min. The onset of action of digoxin after the oral administration in man usually is evident in 1 to 2 h (10). Although the effect of digoxin given orally depends upon the speed of absorption which can be retarded by the presence of food in the gastrointestinal tract and by delayed gastric emptying, there was good agreement between the results in man and in guinea pigs even though the animals were given food and water *ad libitum* in this experiment.

It is clear from the results obtained in the present study that β -methyl-digoxin is superior to digoxin in the speed of intestinal absorption.

Dipyridamole (5 mg/kg, p.o.) also potentiated significantly the adenosine response in guinea pigs (Fig. 3), and the time of onset of its action was 25 min. Jageneau and Schaper (11), who used the microcatheter method in unanesthetized dogs, observed about 30 min after the oral administration of dipyridamole a distinct increase in the coronary blood flow which indicated the onset of action. Beisenherz *et al.* (12), showed that the plasma concentration of dipyridamole significantly increased from 30 min after the oral administration in rats. The time (25 min) of onset of action of dipyridamole administered p.o., estimated by our method, is in good agreement with those values.

It is important to know the time required for cardiac glycosides to act after oral administration. In acute animal experiment, determining the speed of onset of action of these drugs administered p.o. requires a long time of observation and complicated preparations. It was found in the present study that adenosine, infused

i.v., definitely produced heart block which remained constant for 2 to 3 h. This method was applied to the determination of the onset of action of cardiac glycosides administered p.o. in guinea pigs.

It is evident that our method is rather simple but useful in determining the onset of action of these drugs which potentiate the adenosine response.

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