

各種動物の摘出回腸縦走筋標本におけるプロスタグランジン E2の収縮について

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The Prostaglandin E₂-induced Contraction in the Ileal Longitudinal Smooth Muscle Isolated from Various Animal Species

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Abstract. PGE₂ was examined for the effect on contractile response of the ileal longitudinal muscle isolated from eight animal species, monkey, dog, cat, rabbit, guinea pig, vole, rat and mouse. In isotonic recording, PGE₂ induced a sustained contraction in the ileum isolated from monkey and cat. It caused a transient contraction followed by a relaxation in the ileum isolated from other animal species. The sensitivity of ileal strips to the contractile effect of PGE₂, as estimated from 50% effective dose (ED₅₀) of PGE₂, was in the order of guinea pig > vole > rabbit > rat > mouse > monkey > dog > cat. On the other hand, ED₅₀ values of acetylcholine (ACh) were in the concentrations ranging from 1 × 10⁻⁷ M to 5 × 10⁻⁷ M except for guinea pig. Thus, there were species differences in the sensitivity of the ileal strips to PGE₂ but not to ACh. Further, the contractile responses to PGE₂ in the intestine from these animal species were divided into three groups using various inhibitors. In the group 1 animals consisting of herbivorous like rabbit, guinea pig and vole, PGE₂ contractions were inhibited by TTX, atropine, scopolamine or SC-19220. In the group 2 animals consisting of carnivorous like dog and cat, PGE₂ contractions were inhibited by scopolamine and SC-19220 but not by atropine or TTX. In the group 3 animals consisting of omnivorous like monkey, rat and mouse, PGE₂ contractions were inhibited only by SC-19220.

It is known that prostaglandins (PGs) are one of the physiologically active substance in alimentary canal [4, 5, 10, 24]. A very small amount of E type and F type PGs has potent actions on the intestinal smooth muscle [2, 7, 9]. However, the actions are variable depending on the animal species [2, 13] and also on the parts of intestinal tract [1, 2]. That is, PGE type and PGF_{2α} induced a contraction in the ileum isolated from guinea pig [2, 7, 21], human [2], rat [2, 7] or rabbit [3] and colonic longitudinal muscle from guinea pig [1], human [23],

rat [9] or dog [23]. In contrast, PGE₁ and PGE₂ [2, 9, 12] relaxed the circular muscle of ileum and colon of guinea pig, human, rat or dog, although PGF_{1α} and PGF_{2α} [7, 12, 14, 23] contracted them. PGs also stimulated the intrinsic cholinergic nerves in the ileum of guinea pig [2] although they had little effect on neuronal site in the stomach and colonic circular muscle layer of guinea pig [1], rat [11] or human [5].

In the present paper, we examined the contractile response of the ileal longitudinal muscle isolated from eight animal species

to PGE₂, which is involved in a motion of intestinal smooth muscle under physiological conditions [15]. Sensitivities to contractile effect of PGE₂ as well as acetylcholine (ACh) were compared in 50% effective dose. Further, influences of various antagonists on a PGE₂-induced contraction were investigated.

Materials and Methods

The experimental animals used were crab-eating monkey (monkey: ♂ ♀, 1.5-3 kg body weight) dog (♂, approximately 10 kg), cat (♂, approximately 3 kg), rabbit (♂, 2-3 kg), guinea pig (♂, 400-500 g), vole (♂, 40-60 g), rat (♂, 300-400 g) and mouse (♂, 20-35 g). All mammalia were sacrificed by a blow on the neck. After exsanguination, the abdomen were opened and the lower part of the ileum were excised from them. The ileal longitudinal muscle preparations were made as the method described by Paton and Zar [16]. The muscle preparations were suspended in an organ bath containing a modified Tyrode solution of the following composition (mM): NaCl 136.8, KCl 2.7, CaCl₂ 1.8, MgCl₂ 1.0, NaHPO₄ 0.4, NaHCO₃ 11.9 and glucose 5.5, bubbled with 95% O₂ and 5% CO₂ mixture and kept at 36 ± 1°C and pH 7.2.

The contraction of the preparations were recorded isotonicly on a smoked paper using kymograph. Resting tension of 1 g was loaded to the strips isolated from monkey, dog, cat and rabbit, and 0.5 g tension was applied to the preparations isolated from other animal species. The magnification was ten times. The muscle preparations were suspended in organ bath for approximately one hour until the tonus of the ileum became steady. Then 40 mM KCl (40 K) was added hypertonicly to the Tyrode solution. The maximal shortening of the ileum induced by 40 K was attained usually within 10 minutes. In the preparations which showed a sustained contraction induced by PGE₂, cumulative dose-response curves for PGE₂ were obtained. In the preparations which showed a transient contraction induced by PGE₂, each dose of PGE₂ was separately applied to obtain the dose-response relationship. A regression line was fitted by the least squares method. As the maximal shortening of the

ileum was obtained either with 1 × 10⁻⁵ M ACh or with 40 K in all the species, the maximal contraction induced by 40 K was regarded as 100% and the size of contractions induced by PGE₂ or ACh were expressed as a relative contraction (%) to the 40 K-induced contraction.

The effects of various antagonists were examined on the contraction induced by PGE₂ at a concentration close to ED₅₀. An antagonist was usually applied 15 minutes before the application of PGE₂. SC-19220 was solved in ethanol and applied using micropipette. The same volume of ethanol was applied to each ileal preparation in order to confirm that ethanol had no influence upon the preparation.

Drugs: The following drugs were used for the experiment; acetylcholine chloride (ACh; Dai-ichi Seiyaku Co.), atropine sulfate (Wako Co.), scopolamine hydrochloride (Sigma Co.), hexamethonium chloride (C₆; Nakarai Kagaku Co.), prostaglandin E₂ (PGE₂; Ono Yakuhin Co.), 1-acetyl-2-(8-chloro-10,11-dihydrodibenz [b,f] [1,4] oxazepine-10-carbonyl) hydrazine (SC-19220; Searle & Co.) and tetrodotoxin (TTX; Sankyo Co.).

Results

- 1) The contractions induced by 40 K, ACh and PGE₂ in the ileal muscle isolated from various animal species

The ileal longitudinal muscle preparations showed a rhythmic spontaneous contraction in monkey and rabbit, small spontaneous contraction in dog, cat, guinea pig and vole and no spontaneous activity in mouse.

40 K: When 40 K was applied, the ileal strips isolated from monkey and dog shortened rapidly reaching a maximum level within 5 minutes and kept the level for more than 10 minutes (Fig. 1). On the other hand, the muscle from guinea pig and mouse shortened rapidly by 40 K solution, slightly relaxed and a sustained contraction followed (Fig. 1). In rat and vole, the pre-

Fig. 1. Contractile responses to 40 mM KCl and PGE₂ in ileal longitudinal muscle of monkey, dog, cat, rabbit, guinea pig, vole, rat and mouse.

The concentrations of PGE₂ were selected for approximately 50% shortening of that induced by 40 mM KCl.

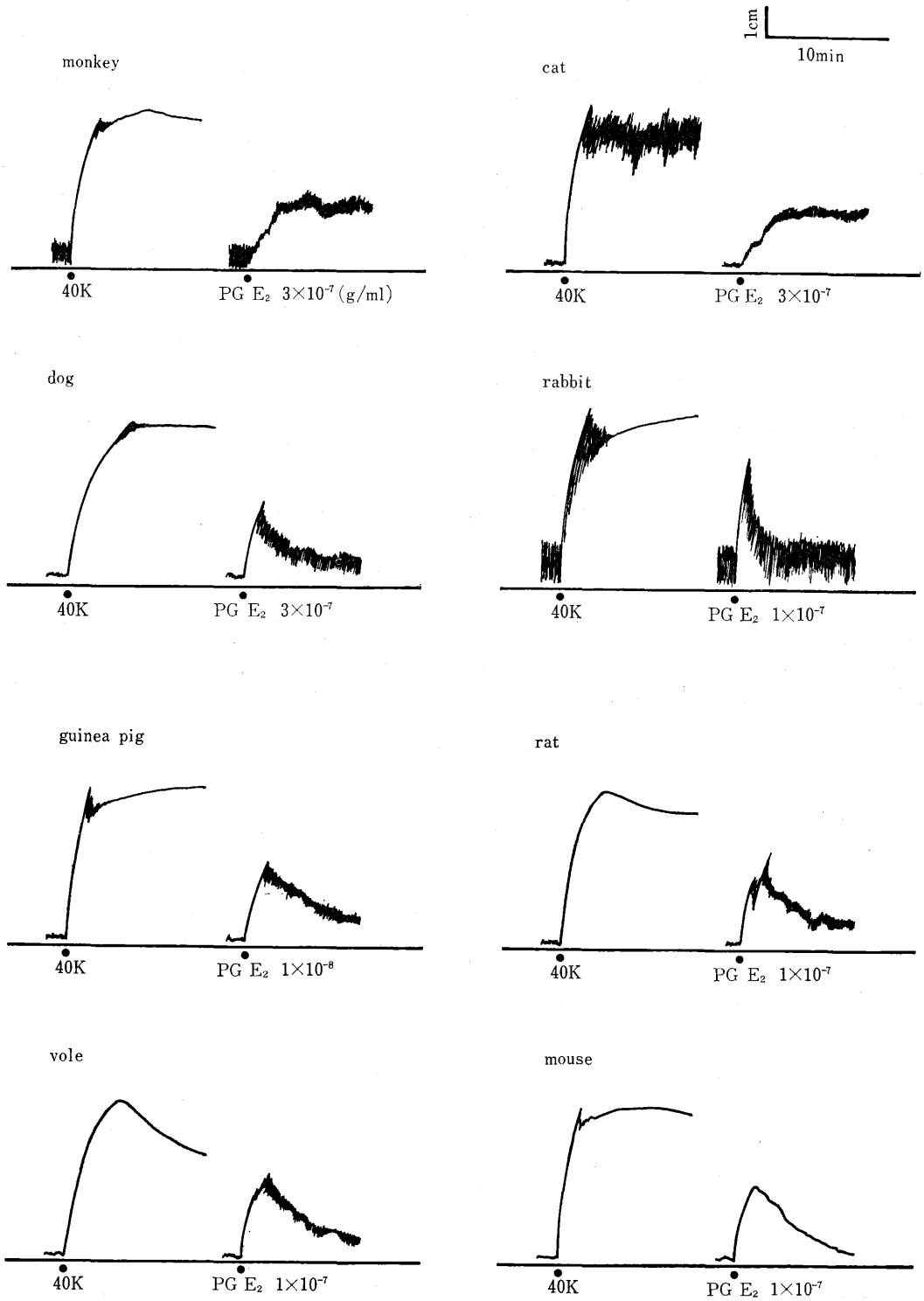


Table 1. Contractile responses to PGE₂ and ACh in ileal longitudinal muscles isolated from various animal species

	Relative contraction induced by 1×10^{-5} M ACh (n)	ED ₅₀ for ACh-induced contraction (M)	Relative contraction induced by 1×10^{-6} g/ml (or 3×10^{-7} g/ml) PGE ₂ (n)	ED ₅₀ for PGE ₂ -induced contraction (g/ml)
Guinea pig	106 ± 1.4% (6)	1.8×10^{-8} (1.6×10^{-8} – 1.9×10^{-8})	*89 ± 3.0% (6)	7.9×10^{-9} (7.1×10^{-9} – 8.5×10^{-9})
Vole	99 ± 3.5% (4)	1.6×10^{-7} (8.7×10^{-8} – 2.3×10^{-7})	*83 ± 3.0% (4)	6.3×10^{-8} (6.2×10^{-8} – 6.7×10^{-8})
Rabbit	122 ± 2.9% (5)	1.3×10^{-7} (1.2×10^{-7} – 1.4×10^{-7})	69 ± 5.0% (4)	7.9×10^{-8} (7.3×10^{-8} – 8.8×10^{-8})
Rat	100 ± 1.3% (8)	1.6×10^{-7} (1.2×10^{-7} – 2.1×10^{-7})	*66 ± 3.8% (7)	7.9×10^{-8} (7.4×10^{-8} – 9.4×10^{-8})
Mouse	108 ± 6.7% (6)	4.0×10^{-7} (3.2×10^{-7} – 4.3×10^{-7})	100 ± 10.4% (8)	8.3×10^{-8} (8.0×10^{-8} – 8.6×10^{-8})
Monkey	102 ± 1.3% (4)	1.6×10^{-7} (8.7×10^{-8} – 2.3×10^{-7})	94 ± 6.9% (6)	1.6×10^{-7} (1.5×10^{-7} – 1.8×10^{-7})
Dog	99 % (3)	—	58 ± 6.1% (4)	5.0×10^{-7} (4.6×10^{-7} – 9.7×10^{-7})
Cat	116 % (2)	—	48 ± 5.9% (4)	> 1.3×10^{-6}

Relative contractions were induced by 1×10^{-5} M ACh or 1×10^{-6} g/ml (or 3×10^{-7} g/ml) PGE₂ and the results are expressed as percentage (%) of the maximal shortening induced by 40 mM KCl. The range of ED₅₀ was calculated from the regression line.

paration immediately shortened to a maximum level within 3 minutes of application of 40 K and then slowly relaxed (Fig. 1). When exposed to 40 K, the ileum of cat exhibited a rapid shortening followed by a sustained shortening superimposed by rhythmic contractions on it (Fig. 1). In rabbit, 40 K increased the amplitude of spontaneous contraction simultaneously with an increase in muscle tonus. When the muscle maximally shortened, the spontaneous contraction disappeared and the muscle shortening remained at a steady level (Fig. 1).

ACh: When exposed to ACh, all muscles isolated from the eight species exhibited a rapid shortening followed by a sustained contraction. The maximum shortening induced by 1×10^{-5} M ACh was almost the same as that induced by 40 K in the ileum of all species (Table 1). The ACh-induced contractions showed a dose-response relationship and ED₅₀ values for ACh were estimated to be in a narrow range from

1×10^{-7} M to 5×10^{-7} M in all the species except guinea pig (Table 1). From these data, it is unlikely that there is species differences in the sensitivity of ileal longitudinal muscle to the contractile effect of ACh.

PGE₂: Contractions induced by PGE₂ at concentrations close to ED₅₀ are shown in Figure 1. In the ileum of monkey, low concentration of PGE₂ (1×10^{-8} g/ml) gradually shortened the preparation accompanied by rhythmic contractions and reached a maximum level within 5 minutes, then maintained it. An application of PGE₂ at a concentration close to ED₅₀ (1×10^{-7} – 3×10^{-7} g/ml) shortened the muscle to a maximum level within 3 minutes and maintained the level which is accompanied by rhythmic contractions (Fig. 1). A higher concentration of PGE₂ (1×10^{-6} g/ml) immediately shortened the muscle and then gradually relaxed. A dose-response curve was obtained by a cumulative application of PGE₂ and calculated ED₅₀ was 1.6×10^{-7}

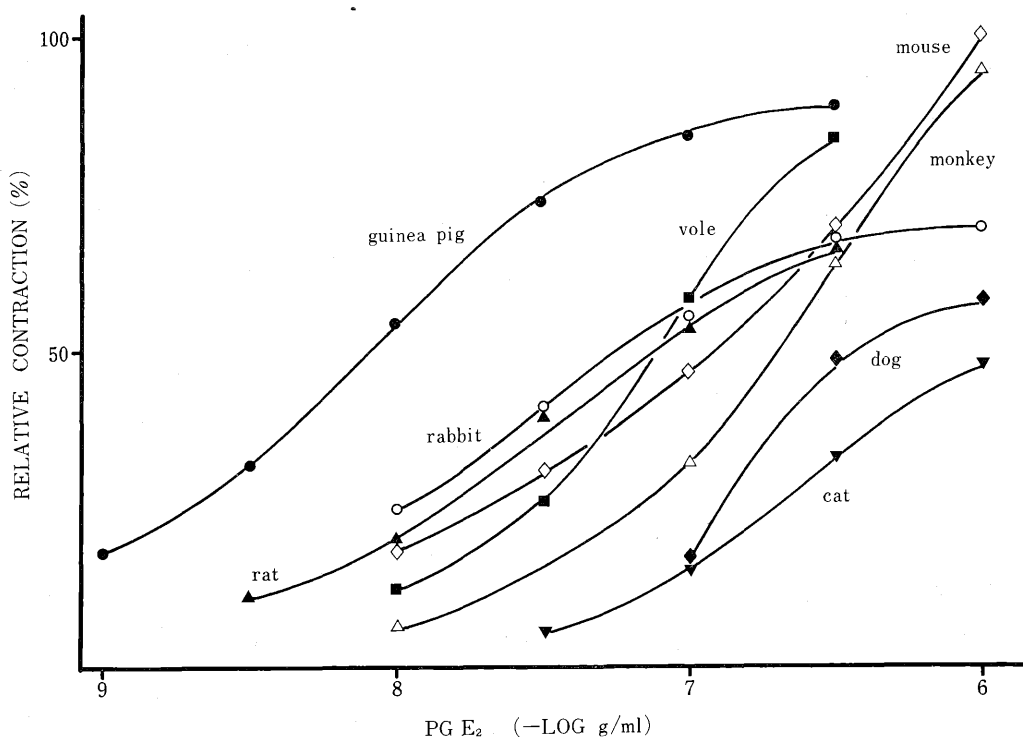


Fig. 2. Dose-response curves of PGE₂ in ileal longitudinal muscle isolated from various animal species.

Ordinate: Relative contraction (%) to the maximum contraction induced by 40 mM KCl. Abscissa: PGE₂ concentration in $-\log$ g/ml. Each experimental point represents the mean value of 4-8 determinations.

g/ml (Fig. 2 and Table 1).

In the ileum from cat, the application of PGE₂ (3×10^{-8} – 1×10^{-6} g/ml) slowly shortened the muscle accompanied with spontaneous contraction, reaching a maximum level within 5 minutes and maintained the level (Fig. 1). A dose-response curve was obtained by single applications of PGE₂. Since the highest concentration of PGE₂ used (1×10^{-6} g/ml) did not induced maximum contraction in cat ileum, precise ED₅₀ value was not obtained except to suggest that the value is above 1.3×10^{-6} g/ml (Fig. 2 and Table 1).

In the ileum of dog, rabbit, guinea pig and rat, an application of PGE₂ (1×10^{-9} – 1×10^{-6} g/ml) immediately induced a maxi-

imum shortening and then gradually relaxed it. Rhythmic contraction was induced during the slow relaxation. In mouse, however, the ileum did not show PGE₂-induced rhythmic contraction (Fig. 1). As PGE₂ produced only a transient response in these animal species, dose-response curves were obtained by single applications of PGE₂. ED₅₀ values for PGE₂ were 5.0×10^{-7} g/ml, 7.9×10^{-8} g/ml, 7.9×10^{-9} g/ml, 7.9×10^{-8} g/ml and 8.3×10^{-8} g/ml in dog, rabbit, guinea pig, rat and mouse, respectively (Fig. 2 and Table 1).

In the ileum of vole, a low concentration of PGE₂ (1×10^{-8} – 3×10^{-8} g/ml) induced a rapid contraction reaching a maximum level within 2-3 minutes and then slow relaxa-

Table 2. Effects of TTX, atropine, scopolamine and SC-19220 on the contraction induced by PGE₂ in ileal longitudinal muscle isolated from various animal species

	TTX 1×10 ⁻⁷ g/ml	Atropine 1×10 ⁻⁶ M	Scopolamine 1×10 ⁻⁶ M	SC-19220 3×10 ⁻⁵ M
Rabbit	+	+	⊕	⊕
Guinea pig	⊕	⊕	⊕	⊕
Vole	⊕	⊕	⊕	⊕
Dog	—	—	⊕	⊕
Cat	—	—	⊕	⊕
Monkey	—	—	—	⊕
Rat	—	—	—	⊕
Mouse	—	—	—	⊕

Inhibition: — No effect, + <25%, ⊕ >25%.

PGE₂-induced contraction was not influenced by hexamethonium (1×10⁻⁴ M).

tion accompanied by rhythmic contraction (Fig. 1). A sigmoidal dose-response curve was obtained by non-cumulative applications of PGE₂ and ED₅₀ was 6.3×10⁻⁸ g/ml (Fig. 2 and Table 1).

The maximum effect of PGE₂ was obtained by the application of PGE₂ in the concentration below 1×10⁻⁶ g/ml in the ileum dissected from all the animal species examined except for cat. The maximum effect of PGE₂ was nearly the same as that induced by 40 K in monkey, guinea pig, vole and mouse, suggesting that PGE₂ acted as a full agonist in the ileum of these animal species. In rabbit, rat, dog and cat, however, the maximum effect of PGE₂ was smaller than that of 40 K, suggesting that PGE₂ acted as a partial agonist.

These results indicate that the sensitivity to PGE₂ of the guinea pig ileum was the highest and the sensitivity decreased in the order of vole, rabbit, rat and mouse. The longitudinal muscle strips isolated from monkey, dog and cat were the least sensitive to PGE₂. Therefore, it seems that there are species differences in the sensitivities of ileal longitudinal muscles to a contractile effect of PGE₂.

2) Effects of several antagonists on the ileal

contraction induced by PGE₂

Experiments were performed to examine effects of several antagonists on the PGE₂-induced contraction in the ileal muscle. The following antagonists were used: TTX, C₆, atropine, scopolamine and SC-19220; a specific PGs antagonist [6, 19, 20]. The PGE₂-induced contraction (1×10⁻⁷ and 3×10⁻⁷ g/ml) were inhibited by SC-19220 (3×10⁻⁵ M), but not by other antagonists in the ileum dissected from monkey and rat respectively. In the ileum of dog and cat, the contraction was inhibited by SC-19220 (3×10⁻⁵ M) and scopolamine (1×10⁻⁶ M). In the rabbit ileum, the contraction was markedly inhibited by SC-19220 (3×10⁻⁵ M) and scopolamine (1×10⁻⁶ M) and slightly inhibited by TTX (1×10⁻⁷ g/ml) and atropine (1×10⁻⁶ M), but not by C₆ (1×10⁻⁴ M). In the ileum of guinea pig and vole, the contraction was remarkably inhibited by TTX (1×10⁻⁷ g/ml), atropine (1×10⁻⁶ M), scopolamine (1×10⁻⁶ M) and SC-19220 (3×10⁻⁵ M), but not by other drugs. In the mouse ileum, the contraction was inhibited by C₆ (1×10⁻⁴ M) and SC-19220 (3×10⁻⁵ M) but not by other antagonists. These results are summarized in Table 2.

Discussion

E type of PGs generally stimulates the longitudinal muscle of the isolated digestive tract [1, 2], but it inhibits the contraction of circular muscle [2, 9, 12]. As it has been reported that the length of longitudinal muscle layer of intestine is affected by the tonus of the circular muscle layer [22], the use of whole ileal preparation was seemed to be unsuitable to examine the effects of PGE₂ on the length of the longitudinal layer. Therefore, in the present paper, the ileal longitudinal muscle preparations dissected from the ileum of various animal species were employed to observe the sensitivity to PGE₂.

The ileal strips isolated from different animal species showed different sensitivity to PGE₂. The ileum from guinea pig was the most sensitive, those from vole, rabbit, rat and mouse were less sensitive and those from monkey, dog and cat were the least sensitive. Bennett et al. [2] have also reported that isolated guinea pig intestine is more sensitive than that of rat and human to the stimulatory effect of PGs. Such species differences were not observed in the sensitivity of ileum to ACh. It is well known that there are species differences in the sensitivity of the digestive tract to the contractile effects of some agents. The intestinal muscle of guinea pig shows higher sensitivity to histamine than that of rat although the latter preparation is more sensitive to 5-hydroxytryptamine (5-HT) than the former [8, 17, 18]. Ouabain [21, 22] and a removal of external potassium from the medium (K removal, unpublished observation) induced a contraction in the small intestine isolated from several animals. The sensitivity to ouabain was the highest in the intestine from frog, pigeon and cock, moderate in those from rabbit,

vole and guinea pig, low in those from rat and mouse, and insensitive in the toad intestine. Similar species differences were observed in the sensitivity to the K removal. Thus, there seems to be different types of species differences in the sensitivity of isolated ileum to various stimulants.

In the ileum of the herbivorous animals (rabbit, guinea pig and vole), the PGE₂-induced contraction was inhibited by TTX, a compound that selectively blocks axonal conduction, and atropine and scopolamine, highly selective antagonists of muscarinic agents. The PGE₂-induced contraction of ileum from the carnivorous animals (dog and cat), was inhibited by scopolamine, but not by TTX or atropine. In the omnivorous animals (monkey, rat and mouse), the PGE₂-induced contraction was not inhibited by TTX, atropine or scopolamine. In all the species examined, PGE₂-induced contraction was inhibited by SC-19220, a specific PGs antagonist [6, 19, 20], but not by C₆, a ganglionic blocker, except for mouse ileal contraction which was inhibited by C₆.

In conclusion, there were species differences in the sensitivity of the ileal longitudinal muscle preparation to the contractile effect of PGE₂. The ileum of guinea pig was the highest sensitive, those of vole, rabbit, rat and mouse were less sensitive, and those of monkey, dog and cat were the least sensitive. Further, it was found that the ilea isolated from herbivorous (rabbit, guinea pig and vole), carnivorous (dog and cat) or omnivorous (monkey, rat and mouse) were differently affected by various antagonists.

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要 約

各種動物の摘出回腸縦走筋標本におけるプロスタグランジン E₂ の収縮について：神崎淳二・清水一政・中條真二郎（日本獣医畜産大学家畜薬理学教室），浦川紀元（東京大学農学部家畜薬理学教室）——プロスタグランジン類（PGs）は広く生体内に存在するが，特に消化管に対する PGs の作用は PGs の種類，実験動物種，消化管の部位等によってまちまちである．本実験は，各種動物（サル，イヌ，ネコ，ウサギ，モルモット，ハタネズミ，ラットおよびマウス）の摘出回腸縦走筋標本を用いて，PGE₂ の収縮反応をマグヌス法によって求め，動物種属間の感受性の差を対比すると共に，各種拮抗薬の影響を観察した．サルおよびネコの回腸縦走筋は PGE₂ により持続性に収縮し，他のものは一過性の収縮後弛緩した．回腸縦走筋標本における各種動物の感受性は濃度作用曲線より，モルモットが最も高く，続いてハタネズミ，ウサギ，ラット，マウスの順に高く，サル，イヌおよびネコは低く，PGE₂ 収縮には種属間に感受性の差が認められた．さらに，PGE₂ 収縮に対する各種拮抗薬の影響を検討したところ，ウサギ，モルモットおよびハタネズミなどの草食動物における PGE₂ 収縮は TTX，アトロピン，スコポラミンおよび SC-19220 で抑制された．またイヌおよびネコの肉食動物においてはスコポラミン，SC-19220 で抑制されたが TTX，アトロピンでは抑制されなかった．そしてサル，ラットおよびマウスの雑食動物においては SC-19220 のみにより抑制された．以上のように，各種拮抗薬の効果から PGE₂ 収縮は 3 つのグループに分けられた．