DIFFERENCE BETWEEN INHIBITORY ACTIONS OF PAPAVERINE AND DEHYDROEPANDROSTERONE ON THE ISOLATED GUINEA-PIG ILEUM

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Abstract—Dehydroepiandrosterone (DEA), a metabolite of steroid hormone in the urine, differed from papaverine in the mode of inhibitory action on the isolated guinea-pig ileum. Papaverine inhibited both phasic and tonic contractions induced by an agonist, while DEA inhibited the phasic contraction without apparent fatigue of the tonic phase. The main action of papaverine seems to be predominantly a metabolic inhibition similar to that of metabolic inhibitors, but the action of DEA could not be ascribed to that of metabolic inhibition. In the presence of papaverine or metabolic inhibitors the height of maximal contraction of an agonist tested by the cumulative method was greatly depressed and less than that by a single method. The longer the time involved in the cumulative doses, the lower was the maximal contraction.

It has been reported by Ishida et al. (1, 2) that in human urine the metabolites of steroid hormones such as dehydroepiandrosterone, androsterone and estrogens show non-specific inhibitions against the response of isolated smooth muscles to several stimulant drugs. Santi et al. (3) pointed out that different mechanisms of action might be involved in the spasmolytic activity of the main papaverine derivatives. With guinea-pig ileum and rabbit duodenum, papaverine mimics the effect of anoxia, cyanide, 2,4-dinitrophenol (2,4-DNP) or other enzyme inhibitors, by suppressing the "tone (tonic) phase" of acetylcholine (ACh), histamine and barium chloride-induced contractions without affecting the "spike (phasic) phase", whereas eupaverine failed to give such the "anoxia-like" effect.

The present study on the isolated guinea-pig ileum is mainly concerned with re-examination of effects of metabolic inhibition on contractions induced by ACh and histamine in order to clarify the difference between the action of papaverine and dehydroepiandrosterone (DEA). It was also noted that the value of inhibitory parameter of a non-competitive antagonist having metabolic inhibitory properties such as papaverine is unreliable when its inhibitory activity is measured using the cumulative dose method (4).

MATERIALS AND METHODS

A strip of the isolated ileum from guinea-pig (300-400 g) was suspended in the organ bath filled with 10 ml of Tyrode solution, kept at 31 ± 1 °C and bubbled with air. Responses of the tissue to drugs were isotonically recorded with a lever of about 0.5 g weight on a smoked drum. The Tyrode solution used had the following composition; NaCl 8.0, KCl
0.2, CaCl₂ 0.2, MgCl₂ 0.1, NaH₂PO₄ 0.05, NaHCO₃ 1.0 and glucose 1.0 g in a litre.

The technique used here was that dose-response curves of stimulant drugs were made by measuring the responses of the isolated ileum on single dose or cumulative doses increasing the dose without washing out in the presence of an inhibitor in order to estimate the inhibitory activity. Stimulant drugs (agonists) used were mainly ACh and histamine. Atropine sulfate (10⁻⁷ M) was previously added into the organ bath for the assay of histamine and chlorpheniramine maleate (10⁻⁷ M), an anti-histaminic, for the assay of ACh. The condition of anoxia was produced by changing the aeration to bubbling with nitrogen gas for 20 min and glucose free solution used was prepared by omitting glucose from the Tyrode solution. As the normal aerobic response to an agonist may be considered a biphasic phase, it is proposed to term the initial contraction the “phasic (spike) phase” and the succeeding tonal contraction the “tonic phase” according to West et al. (5).

Drugs used were acetylcholine chloride (“Ovisot”, Daiichi Seiyaku Co.), histamine dihydrochloride (Wako Pure Chemical Ind.), atropine sulfate, isoprenaline hydrochloride, papaverine hydrochloride and Imidazole (Tokyo Kasei Co.), chlorpheniramine maleate (Kowa Co.), dehydroepiandrosterone and sodium pyruvate (Merk) and caffeine and aminophylline (Japanese Pharmacopoeia). Other pure chemical reagents were purchased from Wako Pure Chemical Ind.; sodium cyanide (NaCN), sodium azide (NaN₃), 2,4-dinitrophenol (2,4-DNP), sodium arsenite, sodium lactate, sodium acetate, sodium succinate, sodium malonate, sodium fumarate, sodium citrate, D-glucose, D-fructose, D-mannose and D-galactose. The imidazole solution was neutralized with 0.1 N HCl and the emulsified solution of dehydroepiandrosterone (DEA) was prepared with a small amount of gummi arabicum.

RESULTS

**Inhibitory action on phasic and tonic phases of the contraction induced by agonists**

Submaximal contractions induced by ACh of 10⁻¹ M are composed of the initial phasic contraction and the succeeding tonal contraction. After incubation of the ileum with papaverine of 10⁻³ M for 10 min, the phasic contraction induced by ACh was partially inhibited and its tonic phase was abolished (Fig. 1A). But, after incubation with DEA of 5 x 10⁻⁵ M, the phasic contraction was suppressed and the succeeding phase was maintained without apparent fatigue (Fig. 1C). From these results it appears that there is a difference between the inhibitory action of papaverine and DEA. These phenomena in the presence of each inhibitor were also observed on the contraction induced by histamine of 10⁻⁶ M.

At least three types of inhibitory action were observed among spasmylytics, metabolic inhibitors and methylxanthine derivatives. The first was that due to inhibitory action of only tonic contraction as shown by NaCN (3 x 10⁻⁴ M), NaN₃ (5 x 10⁻⁴ M), and rotenone (10⁻⁶ M), glucose free solution and anoxia with nitrogen gas (Fig. 1B); the second was that due to inhibitory action of both phasic and tonic phases as shown by 2,4-DNP (10⁻⁴ M) and papaverine (10⁻³ M). In the third, the phasic contraction was firstly suppressed without
FIG. 1. Three types of inhibitory response to the ACh-contraction on the isolated guinea-pig ileum.
A) Both phasic and tonic phases were inhibited by papaverine and 2,4-DNP, B) Only tonic phase was inhibited by NaCN, NaN₃, rotenone, glucose free and anoxia, C) Phasic phase was inhibited without fatigue of tonic phase by DEA and aminophylline.

significantly affecting the tonic contraction as shown by DEA and aminophylline.

Inhibitory pattern of aminophylline was similar to that of DEA, when they had been tested on the ACh-contraction with their graded doses (Fig. 2).

Effects of substrates on the inhibition of tonic phase in the glucose free solution

The tonic phase of ACh- and histamine-contractions which disappeared after incubation of the glucose free solution were recovered in a few minutes by addition of each 10 mM of substrate; the order of potency of substrates for recovery being pyruvate (76.2%),

FIG. 2. Inhibitory pattern of aminophylline and DEA in their graded doses. Note that contractions are suppressed without any fatigue of the tonic phase.

FIG. 3. Effects of substrates on the inhibition of the tonic phase in the glucose free solution. A (left panel): Effect of pyruvate (10 mM), B (right panel): Effect of succinate (10 mM). See the text for the results.
glucose (74.2%), mannose (32.2%), acetate (34.2%) and lactate (31.8%). On the contrary, succinate, citrate, malonate, fumarate and other monosaccharides such as galactose and fructose did not recover to the inhibitory phase (Fig. 3).

**Effect of pyruvate on the tonic phase inhibited by metabolic inhibitors**

The tonic phase of histamine-contraction which disappeared with NaCN (3 x 10^{-4} M) or 2,4-DNP (10^{-4} M) in the normal Tyrode solution containing atropine of 10^{-7} M could be recovered with pyruvate only, but the inhibition by papaverine, rotenone, NaN_{3} and anoxia could not be recovered by application of any of the substrates. The above recovery action of pyruvate for NaCN or 2,4-DNP was prevented by the preincubation of sodium arsenite of 10^{-3} M (Fig. 4). These phenomena suggest that there are different mechanisms of metabolic inhibition between NaCN or 2,4-DNP and other inhibitors.

![Effect of pyruvate on the tonic phase suppressed by inhibitors](image.png)

**Fig. 4.** Effect of pyruvate on the tonic phase suppressed by inhibitors. The tonic phase of histamine contraction suppressed by NaCN (3 x 10^{-4}M) or 2,4-DNP (10^{-4}M) was recovered by addition of pyruvate (left A), but not in the presence of sodium arsenite (right B).

![Synergistic inhibition between papaverine and DEA on the tonic contraction induced by ACh](image.png)

**Fig. 5.** Synergistic inhibition between papaverine and DEA on the tonic contraction induced by ACh.

A) Isoprenaline (Iso) did not potentiate the inhibitions induced by caffeine (Caf) or papaverine (Pap). B) Papaverine potentiated the inhibition induced by DEA. C) Control of inhibitory response to DEA.
Effect of isoprenaline on the inhibition induced by caffeine, papaverine and DEA to the ACCh-
tonic contraction

If papaverine and caffeine would lead to a decreased contractility of the ileum by means
of the inhibitory effect on phosphodiesterase to cyclic AMP (6, 7), their inhibitory action
could be potentiated by isoprenaline on the guinea-pig ileum as well as on the rat uterus
(11). However, isoprenaline up to $10^{-6}$ M did not show any inhibitory action to the ACCh-
tonic contraction on the ileum and there was no synergistic inhibition with papaverine,
caffeine and DEA (Fig. 5A). After weak inhibition of the tonic contraction with papaverine
of $2 \times 10^{-6}$ M, it was observed that adding DEA of $2 \times 10^{-5}$ M augmented within a few
minutes the inhibitory action induced by papaverine (Fig. 5B).

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**Fig. 6.** Effects of papaverine on the dose-response curves for histamine.

A (left panel): (a) Control of cumulative histamine-contractions, (b), (c) and
(d) Cumulative histamine-contractions in the presence of papaverine of $2 \times
10^{-5}$ M, but times required for the procedure are 2' 10'', 4' 00'' and 2' 10'' respec-
tively, (e) A maximum contraction of histamine with a single dose. B
(right panel): The dose-response curves for histamine from the left panel.

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**Fig. 7.** Effects of NaCN on the dose-response curves for histamine.

A (left panel): (a) Control, (b), (c) and (d) Cumulative histamine-contractions
in the presence of NaCN of $10^{-3}$ M, but times required for the procedure are 1' 00'',
5' 00'', 1' 00'' respectively, (e) A maximum contraction of histamine with a single
dose. B (right panel): The dose-response curves for histamine from the left panel,
Imidazole of $3 \times 10^{-3}$ M not having any effect on the resting ileum could not produce a recovery of the inhibitions induced by papaverine of $5 \times 10^{-6}$ M, DEA of $3 \times 10^{-5}$ M and caffeine of $10^{-4}$ M for 5 min on the ACh-tonic contraction.

**Effects of papaverine on the dose-response curves of agonists**

When dose-response curves of histamine in the presence of papaverine were measured for the estimation of its inhibitory activity, the maximum contraction obtained with the cumulative dose technique was evidently lower than that obtained with the single dose. The longer the time required to make a complete curve, the lower the maximum contraction obtained (Fig. 6). Similar results were obtained in the presence of NaCN ($10^{-3}$ M) by which the tonic contraction of the ileum can be predominantly inhibited (Fig. 7).

**DISCUSSION**

The metabolites of steroid hormones in urine, dehydroepiandrosterone (DEA), androsterone and estrogens, showed non-specific inhibitory actions against contractions of the isolated guinea-pig ileum and isolated rat uterus induced by various agonists (2), but in the present work it was demonstrated that DEA may be different from papaverine in the mode of inhibitory action on the isolated guinea-pig ileum.

Three types of mechanism have already been proposed to explain the inhibitory phenomena of papaverine; the first is due to its effects of metabolic inhibition, by which the contraction induced by an agonist develops while the tonic contraction cannot be maintained (3, 5, 10); the second is due to the increasing cyclic AMP by inhibition of cyclic phosphodiesterase activity in adenylcyclase system (6, 7). Regarding the third, it may be assumed that papaverine decreases the effective concentration of a store of bound calcium in the membrane of muscle cells (8, 9).

As shown in Fig. 1, papaverine and 2,4-DNP inhibited both phasic and tonic contractions at any inhibitory concentration. NaCN, NaN₃ and rotenone inhibited only the tonic contraction at the concentration shown in Fig. 1, however, at a higher concentration both agents inhibited both phases. DEA as well as aminophylline never showed an inhibitory action similar to those of papaverine and metabolic inhibitors even though the concentration was increased to $10^{-4}$ M or $10^{-2}$ M (Fig. 2).

Pfaffman et al. (10) reported that the isometric tonic tension of the guinea-pig teania coli induced by high K-medium was reduced after the preparation had been incubated in glucose-free solution. Following the addition of glucose to the bath, there was a gradual reappearance of tension. In our experiments, the tonic contraction of the guinea-pig ileum induced by ACh or histamine which disappeared after repeated incubation with glucose-free solution was rapidly recovered by the addition of glucose of 10 mM to the organ bath (Fig. 4). In restoring the tonic contraction, the ability of substrates was quite similar to that in the taenia coli, but the most active was pyruvate and the order of potency among other substrates was glucose, mannose, acetate, lactate. Only the tonic inhibition induced by NaCN and 2,4-DNP could be recovered by the addition of pyruvate but not by other substrates, while the inhibition induced by NaN₃, anoxia, papaverine and rotenone could
not be recovered by any of the substrates. It is suggested that a mechanism of metabolic inhibition of NaCN or 2,4-DNP might differ from the mechanism of other inhibitors, in its ability to contribute to inhibition of phosphorylation at substrate levels.

On the isolated rat uterus, Mitznegg et al. (11) reported that the relaxing effect of epinephrine on the contraction induced by oxytocin and calcium ions in calcium-free Tyrode solution was potentiated by theophylline. Takagi et al. (13) also showed, on both the rat uterus and the guinea-pig taenia, that the inhibitory responses to isoprenaline, cyclic AMP and papaverine were potentiated by caffeine and decreased by imidazole. The phenomenon of inhibitory action of papaverine and catecholamines on the guinea-pig ileum differed from those on the rat uterus; there were no synergistic inhibitions between isoprenaline and aminophylline (Fig. 5A), and no antagonism between papaverine or caffeine and imidazole. It should be noted that smooth muscles are not a particularly homogeneous group and caution is required in extrapolating data from rat uterus to guinea-pig ileum. One difference between them may be that contractions of rat uterus are greatly inhibited by catecholamines, for example, even a small concentration of isoprenaline at $2 \cdot 10^{-12}$ M revealed an inhibitory action (14), while on the contrary, the tonic contraction of guinea-pig ileum, especially induced by ACh, was hardly inhibited by isoprenaline even up to $10^{-6}$ M.

As a synergistic inhibition between papaverine and DEA was observed (Fig. 5), the mechanisms between them may be different.

Van Rossum (12) reported the cumulative method for the evaluation of non-competitive antagonists such as papaverine-like compounds; the parameter of which is the $pD_2$ value. In the presence of papaverine, the height of maximal contraction of an agonist tested by the cumulative method was greatly depressed and was less than that by a single method. The longer the time used for the cumulative method, the lower was the maximal contraction. But, in the presence of DEA or methyxanthines, the inhibitory activity by the cumulative method was not significantly different from that by the single method, though the maximal contraction had been non-competitively inhibited, the inhibitory action of DEA could not be ascribed to that of metabolic inhibition and differed from that of papaverine.

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