COMPARISON OF ACTIONS OF PAPAVERINE, ASPAMINOL AND ISOPRENALINE ON ISOLATED RAT UTERUS

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Abstract—Inhibitory responses of the isolated rat uterus to papaverine, Aspaminol and isoprenaline were potentiated by aminophylline that inhibits phosphodiesterase. The inhibitory responses to papaverine and isoprenaline were decreased by imidazole that stimulates phosphodiesterase activity, while the inhibitory response to Aspaminol was little influenced by imidazole. Papaverine strongly inhibited phosphodiesterase from the rat uterus, but Aspaminol did not influence phosphodiesterase activity. These results indicate that papaverine and isoprenaline are mediated through an increase of the intracellular level of cyclic AMP and also indicate that the inhibitory response of the rat uterus to Aspaminol is not concerned with the amount of intracellular cyclic AMP increased by inhibiting phosphodiesterase. Further, the results support the theory that there are two mechanisms for the so-called papaverine-like antispasmodics.

The beta-adrenergic action is thought to be related to the ability of the beta-adrenergic stimulants to increase the intracellular level of adenosine 3', 5'-monophosphate (cyclic AMP) in the smooth muscle (1). Recently evidence is presented that papaverine, a smooth muscle relaxant, is a potent inhibitor of phosphodiesterase in rabbit aorta, rat uterus and guinea pig ileum (2, 3). More recently certain workers (4, 5, 6, 7, 8) have reported that papaverine and isoprenaline increased the intracellular level of cyclic AMP in the intestinal and uterine smooth muscle. These results indicate the possibility that the actions of beta-adrenergic stimulants and papaverine are mediated through an increase of the intracellular level of cyclic AMP.

Takagi, Takayanagi and Fujie (9) suggested that the mechanisms of action for papaverine are different from those for synthetic antispasmodics such as benactyzine and Aspaminol (1, 1-diphenyl-3-piperidinobutanol hydrochloride). This theory was confirmed by Takagi et al. (10). Therefore, we have compared the mode of action of Aspaminol with those of papaverine and isoprenaline.

MATERIALS AND METHODS

Virgin female Wistar rats weighing 150 to 200 g were ovariectomized and allowed to recover more than 5 days before beginning an experiment.

Oxytocin-induced Contractions

An ovariectomized rat was stunned and bled, and the uterine horns were removed.

One horn was suspended in a 10 ml organ bath. The bath was filled with Locke-Ringer's solution, kept at 32°C, and gassed with a mixture of 95% O₂ and 5% CO₂. Locke-Ringer's solution had the following composition: 9.0 g of NaCl, 0.4 g of KCl, 0.2 g of CaCl₂, 0.2 g of MgCl₂, 0.5 g of NaHCO₃ and 0.5 g of glucose in a litre. Uterine contractions were isotonicly recorded by means of a lever loaded with 0.5 g on a smoked drum. A concentration-action curve was cumulatively obtained (11, 12). The contractions are expressed as the percentage of the maximum response and each curve represents the mean of at least six experiments.

To compare the effects of the test drugs on the uterine muscle with those on the intestinal muscle, the taenia from the guinea pig caecum was used in some experiments. The inhibitory effects of Aspaminol and isoprenaline in the presence and absence of caffeine or imidazole on the taenia were investigated. After sacrificing a male guinea pig (300 to 350 g in body wt.) by a blow on the neck, the taenia was isolated from the caecum. A piece of the taenia was suspended in a 10 ml organ bath filled with Locke-Ringer's solution, and kept at 37°C. Other experimental conditions were the same as those used for the rat uterus.

Anti-phosphodiesterase activity of test drugs

The uteri isolated from five ovariectomized rats were homogenized with the 10-fold of a buffer-Mg²⁺-solution (Tris-HCl 16×10^{-2} M, Mg-acetate 5×10^{-3} M; pH 7.5) at 0°C according to the method of Pösch (13). The homogenate was centrifuged at $2,000 \times g$ for 15 min at 0°C and the homogenate fluid was used as enzyme preparation.

Phosphodiesterase activity was assayed according to Pösch (13). The principle of the determination of phosphodiesterase-activity used is based on the decrease in radioactive substrate (³H-cyclic AMP) during incubation with the enzyme. This decrease can be measured since the product of the reaction, labeled 5'-AMP, in contrast to the substrate can be removed from the reaction mixture by ZnSO₄-Ba(OH)₂-precipitation (14). Non-labeled 5'-AMP was added in a high concentration (1 mM) which did not interfere with the phosphodiesterase-reaction since the reaction product, labeled 5'-AMP, is further metabolized in crude enzyme preparations which contain varying amounts of 5'-nucleotidase. Incubation was carried out at 37°C for 30 min with 300 μ l supernatant (corresponding to 12 mg wet wt.), to which 50 μ l of non-labeled 5'-AMP (adjusted to pH 7.5- and 50 or 100 μ l of a test drug-solution were added. The reaction was started by the addition of 50 μ l of the labeled substrate. The reaction volume always 500 μ l. The Michaelis constant (K_m) (15) and inhibitor constant (K_i) (16) were graphically determined.

Drugs used: Papaverine hydrochloride (Tokyo Kasei Kogyo). Aspaminol (1, 1-diphenyl-3-piperidinobutanol hydrochloride, Kowa Co. Ltd.) L-isoproterenol-D-bitartrate (Sigma). Caffeine (Wako Pure Chemical Industries, Ltd.). Aminophylline ((Theophylline)₂-ethylenediamine, Sigma). Imidazole (Tokyo Kasei Kogyo). Oxytocin (Atonin-S, Teikoku Zoki). Cyclic 3', 5'-AMP (adenosine-3', 5'-monophosphate, Daiichi Pure Chemicals, Co. Ltd.). 5'-AMP (adenosine-5'-monophosphate, Daiichi Pure Chemicals, Co. Ltd.). ³H-cyclic 3', 5'-AMP (adenosine-8-labeled, Daiichi Pure Chemicals, Co. Ltd., specific activity 7.7 Ci/mmmole).

RESULTS

Effects of aminophylline and imidazole on inhibitory responses of the rat uterus to Aspaminol, papaverine and isoprenaline

Fig. 1 shows the effects of Aspaminol, papaverine and isoprenaline on the oxytocin-induced contractions in the presence and absence of aminophylline in the isolated rat uterus. The inhibitory actions of isoprenaline (2×10^{-10} g/ml) and Aspaminol (10^{-5} g/ml) were greatly potentiated by aminophylline (5×10^{-5} g/ml) (Fig. 1, A, C). On the other hand, aminophylline (5×10^{-5} g/ml) also potentiated the inhibitory action of papaverine (10^{-6} g/ml) (Fig. 1, B). This potentiation was not so great as those of the actions of isoprenaline and Aspaminol by aminophylline.

In the presence and absence of imidazole the inhibitory responses to Aspaminol, papaverine and isoprenaline are shown in Fig. 2. Imidazole greatly antagonized the responses to isoprenaline (2×10^{-10} g/ml) and papaverine (10^{-6} g/ml) at the concentrations of 5×10^{-5} g/ml and 3×10^{-4} g/ml respectively (Fig. 2, A, B). Imidazole (3×10^{-4} g/ml), however, little influenced the response to Aspaminol (2×10^{-5} g/ml) (Fig. 2, C). Similar results were obtained with caffeine (10^{-4} g/ml).

In the taenia isolated from the guinea pig caecum, the inhibitory response to Aspaminol was little influenced by caffeine (10^{-4} g/ml) or imidazole (10^{-4} g/ml) (Fig. 3, A, B),

FIG. 1. Effect of aminophylline (5×10^{-5} g/ml) on the actions of (A) isoprenaline (2×10^{-10} g/ml), (B) papaverine (10^{-6} g/ml) and (C) Aspaminol (10^{-5} g/ml) on oxytocin-induced contractions.

The rat uterus was incubated with isoprenaline, papaverine and Aspaminol for 3 min respectively. After 5 min incubation of the uterus with aminophylline, each antagonist was added. In their presence, cumulative concentration action curves of oxytocin were obtained.

FIG. 2. Actions of (A) isoprenaline (2×10^{-10} g/ml), (B) papaverine (10^{-6} g/ml) and (C) Aspaminol (2×10^{-3} g/ml) in the presence and absence of imidazole (5×10^{-5} g/ml in (A), 3×10^{-4} g/ml in (B) and (C)).

Incubations were performed with imidazole for 5 min. Other experimental conditions are described in Fig. 1.

although the response to isoprenaline was potentiated by caffeine (10^{-4} g/ml) and antagonized by imidazole (10^{-4} g/ml) as it did in the rat uterus.

Fig. 4 shows the antagonism between aminophylline and imidazole on the inhibitory responses to Aspaminol, papaverine and isoprenaline. It was later confirmed that the inhibitory responses to Aspaminol, papaverine and isoprenaline were potentiated by aminophylline (5×10^{-5} g/ml), those to Aspaminol, papaverine and isoprenaline were again obtained in the presence of both aminophylline (5×10^{-5} g/ml) and imidazole (3×10^{-4} g/ml). The responses to isoprenaline (2×10^{-10} g/ml) and papaverine (10^{-6} g/ml) were greatly reduced by application of both aminophylline and imidazole, while the inhibitory response to Aspaminol was little influenced (Fig. 4. C).

Effects of Aspaminol and papaverine on phosphodiesterase activity

Phosphodiesterase activity was determined by incubating the substrate (10^{-4} M) in the presence of 1 mM 5'-AMP with a 25-fold crude phosphodiesterase at 37°C, pH 7.5, for 30 min. Papaverine at the concentrations of 1, 3 and 10×10^{-5} g/ml decreased phosphodiesterase activity by 16.8, 61.2 and 94.9% respectively and a dose-dependent inhibition was found with papaverine, while Aspaminol did not influence phosphodiesterase activity (Table 1). Moreover, aminophylline potentiated the action of papaverine on phosphodiesterase activity but not on that of Aspaminol (Table 2).

FIG. 3. Effects of (A) caffeine (10^{-4} g/ml) and (B) imidazole (10^{-4} g/ml) on an inhibitory action of Aspaminol in taenia.

The taenia from the guinea pig caecum was incubated with caffeine for 20 min and with imidazole for 10 min. The taenia was relaxed by caffeine and recovered to the original base in 15 to 20 min. After that, the concentration action curves were obtained in the presence of caffeine.

FIG. 4. Antagonism between aminophylline (5×10^{-5} g/ml) and imidazole (3×10^{-4} g/ml) on responses to (A) isoprenaline (2×10^{-10} g/ml), (B) papaverine (10^{-6} g/ml) and (C) Aspaminol (2×10^{-5} g/ml).

The uterus was incubated with both aminophylline and imidazole for 5 min. Other experimental conditions are described in Fig. 1.

TABLE 1. Mean values of percent phosphodiesterase-inhibition by papaverine and Aspaminol and standard error of 3 to 6 estimations.

Drug	Concentration (g/ml) in the reaction mixture		
	10^{-5}	3×10^{-5}	10^{-4}
Papaverine	16.8 ± 2.33	61.2 ± 3.40	94.9 ± 1.92
Aspaminol		0.7 ± 1.87	-0.2 ± 1.20

TABLE 2. Mean values of percent Phosphodiesterase-inhibition by papaverine and Aspaminol in the presence and absence of aminophylline and standard error of 3 estimations.

* P-value was determined by comparing (A) with (B) or (C) with (D).

Drug	Phosphodiesterase-inhibition (%) mean $\pm$ S.E.	P-value*
Papaverine (3×10^{-5} g/ml)	77.5 ± 1.99 (A)	
Aminophylline (10^{-4} g/ml)	36.2 ± 1.05	
Papaverine (3×10^{-5} g/ml) + Aminophylline (10^{-4} g/ml)	84.4 ± 1.68 (B)	<0.05
Aspaminol (10^{-4} g/ml)	-0.9 ± 5.36	
Aminophylline (3×10^{-4} g/ml)	44.9 ± 2.83 (C)	
Aspaminol (10^{-4} g/ml) + Aminophylline (3×10^{-4} g/ml)	44.1 ± 4.58 (D)	>0.05

FIG. 5. Estimations of (A) K_m value for cyclic AMP and (B) K_i value.

Inhibition of phosphodiesterase by papaverine [I] was measured at two substrate concentrations ($\circ$ — $\circ$ 10^{-4} M, $\bullet$ — $\bullet$ 0.5×10^{-4} M) and determined from Dixon plots.

The K_m and K_i values were obtained by incubating the substrate (0.5 and 1.0 mM) in the presence and absence of papaverine or Aspaminol with a 25-fold crude preparation of the esterase at 37°C , pH 7.5, for 30 min. All determinations were carried out in the presence of 1 mM 5'-AMP in the reaction mixture. The K_m value for cyclic AMP was 2.5×10^{-4} M (Fig. 5, A). The K_i value was 7.0×10^{-5} M and the type of inhibition was competitive (Fig. 5, B).

DISCUSSION

Mitznegg, Heim and Meythaler (17) and also Mitznegg, Hach and Heim (18) have shown in isolated rat uterus that exogenously applied cyclic AMP and caffeine inhibit the contractile responses induced by oxytocin, and suggested that caffeine might increase the intracellular content of cyclic AMP by inhibiting phosphodiesterase. Moreover, Takagi, Takayanagi and Tsuchida (19) using the taenia from the guinea pig caecum, have reported that relaxing actions of papaverine and isoprenaline were affected by caffeine and imidazole. In the present study, the inhibitory actions of isoprenaline and papaverine were potentiated by aminophylline, an agent that inhibits phosphodiesterase, and antagonized by imidazole, an agent that stimulates phosphodiesterase activity, and antagonistic relation between aminophylline and imidazole was observed. These results are in good agreement with those reported previously (19). Further, phosphodiesterase from the rat uterus was competitively inhibited by papaverine, therefore it is suggested that non-competitive anti-oxytocin action of papaverine might be exerted through its inhibition on phosphodiesterase. In view of these results, the inhibitory responses to isoprenaline and papaverine might inhibit the oxytocin-induced contractions of the isolated rat uterus by accumulation of cyclic AMP.

The inhibitory response of the isolated rat uterus to Aspaminol was greatly potentiated by aminophylline, while imidazole did not decrease the response to Aspaminol. Further, Aspaminol had little influence on phosphodiesterase activity. Therefore, this potentiation by aminophylline cannot be explained on the basis of accumulation of the intracellular cyclic AMP. The potentiation of the action of Aspaminol by aminophylline may result from its effect on the cell membrane. In view of these results, it can be concluded that the noncompetitive antioxytocin action of Aspaminol is not concerned with the amount of intracellular cyclic AMP increased by inhibiting phosphodiesterase. Takagi, Takayanagi and Fujie (9) and also Takagi et al. (10) divided "papaverine-like" antispasmodics into two groups according to their mechanisms of action; strong basic substances or most synthetic antispasmodics such as Aspaminol belong to the first group and weak bases such as papaverine and neutral substances as isoamylesters belong to the second. The present results support the theory that there are two different mechanisms for the so-called papaverine-like antispasmodics.

The inhibitory response of the taenia to Aspaminol was little influenced by caffeine in contrast to the results obtained with the rat uterus. This difference could be due to species and organ differences.

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