A NEWLY ISOLATED ANTISPASMODIC —BUTYLIDENEPHTHALIDE—

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Abstract—Butylidenephthalide (BdPh), ligustilide and butylphthalide were isolated and purified from neutral oil of *Ligusticum wallichii* Franch. Among these three, BdPh proved to be the most active in inhibiting rat uterine contractions induced by prostaglandin $F_{2\alpha}$, oxytocin and ACh. In studies done to compare the effects of BdPh and papaverine (Pap), guinea pig ileum, vas deferens and taenia coli were used. BdPh inhibited contractile responses of the ileum to agonists including ACh, K^+ and Ba^{2+} in normal Tyrode solution and to exogenous Ca^{2+} in high K^+ (80 mM), Ca^{2+} -free Tyrode solution, and also responses of vas deferens responses to norepinephrine. Thus, BdPh is a non-specific antispasmodic but weaker in potency than Pap. However, as the inhibitory effects of BdPh on phasic contraction (PC) and tonic contraction (TC) of preparations, including depolarized and non-depolarized ileum and taenia coli, were much the same, it is suggested that the action mechanism of BdPh may differ from that of Pap which inhibited TC more selectively than PC. It may be concluded that BdPh possesses an non-specific antispasmodic action like Pap, the mechanism of action being different from that of Pap.

The rhizome of *Ligusticum wallichii* Franch (Umbelliferae), has been used by the Chinese for several thousands of years to relieve head and stomach pain. From the neutral oil of this crude drug we have already isolated and purified three antispasmodics, butylidenephthalide (BdPh), ligustilide and butylphthalide (1, 2). Among these three, BdPh proved to be the most active in inhibiting rat uterine contractions induced by prostaglandin $F_{2\alpha}$ (1), oxytocin and acetylcholine (ACh) (3). In the experiments reported herein, BdPh only was used.

To elucidate the pharmacological action mechanism of BdPh we compared the effects of this substance on the guinea pig ileum, vas deferens and taenia coli with those of papaverine (Pap).

MATERIALS AND METHODS

Chemicals: Butylidenephthalide (BdPh) was isolated and purified from the neutral oil of rhizome of *Ligusticum wallichii* Franch according to the method described by Bohrmann et al. (4) and modified by Ko et al. (1, 2). The chemical structure shown in Fig. 1 was confirmed by infrared, nuclear magnetic and fragmentary spectra (2). The other chemicals used in this study were papaverine hydrochloride (Dainippon Pharmaceutical

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Co., Ltd.), acetylcholine hydrochloride (Ovisot, Daiichi Seiyaku Co., Ltd.), dl-norepinephrine hydrochloride (NE, Sankyo Co., Ltd.) potassium chloride (Wako Pure Chemical Industries Ltd.), barium chloride (Wako Pure Chemical Industries Ltd.) and calcium chloride (Kokusan Kagaku Co., Ltd.).

All the concentrations of these chemicals were expressed in molarity. However, the final concentration in medium of alcohol, used as a solvent for BdPh, was 0.2%.

Guinea pig ileum: From male guinea pigs weighing 400–450 g, terminal sections of the ileum about 2.5 cm in length were prepared and mounted in 15 ml of normal or high K^+ (80 mM), Ca^{2+} -free Tyrode solution, oxygenated with 95% O_2 –5% CO_2 and maintained at 37°C, and responses of the tissues were recorded isometrically with an initial tension of 0.5 g. The normal Tyrode solution consisted of the following composition (mM): NaCl, 137; KCl, 2.7; $MgCl_2$, 1.0; NaH_2PO_4 , 0.42; $CaCl_2$, 1.8; $NaHCO_3$, 11.9; dextrose, 5.55, and high K^+ (80 mM), Ca^{2+} -free Tyrode solution consisted of the above composition with KCl, 80 mM instead of 2.7 mM, but without $CaCl_2$. The agonists were cumulatively added to the bath (5) and the log dose response relationships were obtained. All antagonists were applied 2 min before adding the agonists to the bath.

Guinea pig vas deferens: The vas deferens from male guinea pigs weighing 300–400 g was mounted in 15 ml of low Mg^{2+} -Tyrode solution, the composition being the same as that of normal Tyrode solution except that $MgCl_2$ 0.1 mM instead of 1.0 mM was included in order to increase the sensitivity to the agonist, NE. The solution was kept at 37°C, oxygenated with 95% O_2 –5% CO_2 , and the agonist was cumulatively added to the organ bath (5). The log dose response relationships were then obtained. The contraction of the preparation was recorded isotonicity with an initial tension of 0.5 g. All antagonists were applied 2 min before adding NE to the bath.

Guinea pig taenia coli: From a male guinea pig weighing 300–400 g, strips of taenia coli, approx. 3–5 cm long, were isolated and mounted in 15 ml of barbital-buffered Tyrode solution (6), which was oxygenated with 95% O_2 –5% CO_2 , and kept at 37°C. After an initial tension of 2 g was applied to the preparation, all the experiments were performed according to the method described by Pfaffman and McFarland (6). Preparations had been treated for 15 min with BdPh, before the contractile response to agonists was obtained. Isometric contractions were recorded via a force-displacement transducer (Nihonkoden Kogyo Co.) on an ink-writing oscillograph.

The pID_{50} was considered to be equal to the negative logarithm of concentrations of antagonists at which a half-maximal inhibitory effect was produced.

Statistical analysis: Tension changes or contraction responses produced by agonists in the presence of antagonists were reported as a percentage of the control tension or responses to the agonists obtained in the absence of antagonists. The calculation of pD_2'

FIG. 1. Chemical structure of butyldenephthalide (BdPh), isolated from *Ligusticum wallichii* Franch. (m. wt. 188)

was performed according to the method described by Ariëns and Van Rossum (7). Statistical significance was determined using Student's *t*-test.

RESULTS

Effects of BdPh and Pap on guinea pig ileum in normal Tyrode solution: Figures 2, 3 and 4 illustrate the inhibitory effects of BdPh (left) and Pap (right) on the log dose response curves of ACh, KCl and BaCl₂, respectively. BdPh non-competitively inhibited the contractions induced by these agents to the same extent as Pap. The pD_2' values of BdPh obtained from the curves of these agents were significantly ($p < 0.001$) different from those of Pap.

Figure 5 shows the inhibitory effects of BdPh and Pap on the PC and TC induced by ACh, KCl and BaCl₂. Pap inhibited TC more selectively than PC, while the inhibition by BdPh was much the same.

Effects of BdPh and Pap on guinea pig ileum: Figure 6 illustrates the inhibitory effects of BdPh on the PC (left) and TC (right) induced by the addition of calcium ion while Fig. 7 shows the effects of Pap. BdPh as well as Pap non-competitively inhibited the response to

FIG. 2. Inhibitory effects of butylidenephthalide (BdPh) and papaverine (Pap) on ACh induced contractions of guinea pig ileum. Each point and pD_2' values represent mean $\pm$ S.E.M., n =number of experiments.

FIG. 3. Inhibitory effects of butylidenephthalide (BdPh) and papaverine (Pap) on K⁺ induced contractions of guinea pig ileum. Each point and pD_2' value represent mean $\pm$ S.E.M., n =number of experiments.

Ca^{2+} in preparations exposed to Ca^{2+} -free, high K^{+} media. The pD_2' values of BdPh obtained from the curves of PC and TC were not significantly different, while those of Pap were significantly ($P < 0.001$) different.

Effects of BdPh and Pap on guinea pig vas deferens: Figure 8 illustrates the effects of BdPh (left) and Pap (right) on the log dose response curves of NE. BdPh as well as Pap non-competitively inhibited the response to NE. However, BdPh ($1\text{--}2.5 \times 10^{-4}$ M) sig-

FIG. 4. Inhibitory effects of butyridenephthalide (BdPh) and papaverine (Pap) on Ba^{2+} induced contractions of guinea pig ileum. Each point and pD_2' value represent mean $\pm$ S.E.M., n =number of experiments.

FIG. 5. Inhibitory effects of butyridenephthalide (BdPh, 10^{-4} M) and papaverine (Pap, 2×10^{-5} M) on contractile responses of guinea pig ileum to ACh (10^{-6} M), K^{+} (40 mM) and Ba^{2+} (20 mM).

FIG. 6. Inhibitory effects of butyridenephthalide (BdPh) on Ca^{2+} induced phasic (PC, left) and tonic contractions (TC, right) in guinea pig ileum exposed to high K^{+} (80 mM), Ca^{2+} -free Tyrode solution. The pD_2' values of BdPh on PC and TC are not significantly different. Each point and pD_2' value represent mean $\pm$ S.E.M. n =number of experiments.

FIG. 7. Inhibitory effects of papaverine (Pap) on Ca^{2+} induced of phasic (PC, left) and tonic contractions (TC, right) in guinea pig ileum exposed to high K^+ (80 mM), Ca^{2+} -free Tyrode solution. The pD_2' value of Pap on TC is significantly greater ($p < 0.001$) than that on PC. Each point and pD_2' value represent mean $\pm$ S.E.M., n=number of experiments.

FIG. 8. Effects of butylidenephthalide (BdPh) and papaverine (Pap) on NE induced contractions of guinea pig vas deferens. The pD_2' value of Pap is significantly greater ($P < 0.01$) than that of BdPh. Each point and pD_2' value represent mean $\pm$ S.E.M., n=number of experiments. W: After replacement of high concentration of BdPh (5×10^{-4} M) or Pap (1×10^{-4} M) three times with fresh fluids.

nificantly potentiated the contractions induced by NE in a dose range from 10^{-6} M to 10^{-5} M, and BdPh in a high concentration (5×10^{-4} M) significantly potentiated contractions induced by NE in a dose range from 10^{-6} to 3×10^{-6} M. After washout of the preparations exposed to the high dose of BdPh, the potentiation was reversed. The effect of NE was not significantly potentiated by Pap.

Effects of BdPh on guinea pig taenia coli: The pID_{50} values of BdPh for spontaneous contraction, K^+ (40 mM)-induced PC and TC, and K^+ -induced TC were 4.14 ± 0.15 , 3.97 ± 0.31 , 3.87 ± 0.04 and 4.03 ± 0.09 , respectively. These values represent mean $\pm$ S.E.M. of seven experiments, and do not significantly differ from each other.

DISCUSSION

The results of the experiments carried out here suggest that BdPh is a non-competitive, non-specific or papaverine-like antispasmodic. Furthermore, from the pD_2' value of BdPh

to ACh (Fig. 2) is less ($p < 0.001$) than that to K^+ (Fig. 3) and greater ($P < 0.02$) than that to Ba^{2+} (Fig. 4), therefore, BdPh does not selectively act on specific cholinergic sites. The results that the pD_2' values of BdPh to all agonists used were significantly less ($P < 0.001$) than those of Pap indicate that the antispasmodic activities of BdPh are weaker than those of Pap.

From previous observations of responses in the taenia coli, it has been suggested that the phasic component of the K-contraction (8) occurs during a rapid membrane depolarization which releases an intracellular calcium (9). From evidence obtained in uterine tissue (10), it has been pointed out that ACh owes its spasmogenic effects on certain smooth muscle at least in part to a depolarizing action accompanied by an influx of extracellular calcium into the contractile cell. Furthermore, many authors suggest that barium produces its spasmogenic effects primarily by releasing bound calcium from intracellular storage pools (11–15). In addition, Van Breemen et al. suggested that NE initiates aortic contractions by releasing intracellularly sequestered calcium (16). Therefore, the present results suggest that BdPh, like Pap, probably inhibits Ca^{2+} release from cellular membrane (17, 18) and from intracellular calcium storage, and/or inhibits Ca^{2+} influx from extracellular fluid (18). This suggestion is also supported by the finding that BdPh inhibits PC and TC of polarized (Fig. 5) and depolarized (Fig. 6) preparations.

The inhibitory effects of BdPh on PC and TC were not significantly different, while Pap inhibited TC more selectively than PC, suggesting that the mechanism of action of BdPh is different from that of Pap. The inhibitory effect of Pap on TC is attributed to inhibition of mitochondrial respiration (19), while other authors suggest Pap inhibits phosphodiesterase activity (20, 21), as well as other probabilities. Further study is under way to elucidate the mechanism of action of BdPh.

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