DIFFERENCE BETWEEN ADRENERGIC β_1 - AND β_2 -BLOCKING EFFECTS ON ISOPROTERENOL-INDUCED Ca SPIKE SUPPRESSION IN GUINEA-PIG TAENIA COLI

Ikuko KIMURA, Hiroshi KATOH and Masayasu KIMURA

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences,
Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan

Accepted July 15, 1983

Abstract—Ca spike suppressions induced by isoproterenol (IsP) and a β_2 -agonist, 5-hydroxymethyl-6-hydroxy-2-isopropylamino-1,2,3,4-tetrahydronaphthalene-1-ol (AA497), were investigated in the presence of butoxamine or practolol. The relaxations were isotonicity recorded, and the Ca spike frequency was recorded using the single sucrose gap method. IsP-induced relaxation was not inhibited by butoxamine (Butox, 0.16 μ M), but was inhibited by practolol (Prac, 0.188 μ M). In 24 mM K^+ -Krebs' solution, AA497 caused relaxation in a lower dose range and suppressed to a small extent the Ca spike frequency in a higher dose range, as was observed for IsP-induced curves of log dose-spike frequency and log dose-relaxation. In normal K^+ -Krebs' solution, both IsP and AA497 greatly suppressed the Ca spike frequency. IsP (1.21 μ M)-induced suppression of the Ca spike frequency was blocked by Prac (113 μ M), and it was blocked by Butox (96 μ M) to a greater extent. AA497-induced suppression of the spikes was not blocked by Prac (37.6 μ M), but completely blocked by Butox (32 μ M). These selective inhibitory effects of butoxamine on AA497-induced and IsP-induced Ca spike suppression demonstrate that in the adrenergic β -receptor-mediated process in taenia coli, β_2 -mechanisms are more closely related to the Ca spike suppression than the β_1 -mechanisms are.

Since the proposal of Lands et al. (1, 2) gave a clue for finding β_1 -adrenergic receptors in the heart and β_2 -adrenergic receptors in both smooth and skeletal muscles, there is now convincing evidence that the distribution of β_1 - and β_2 -adrenergic receptors varies widely in many mammalian organs and different species (3–6). However, the difference in the electropharmacological mechanism of β_1 - and β_2 -adrenergic reactions has not yet been elucidated. In a previous paper, we succeeded in a quantitative analysis of spike frequency and confirmed that the relaxation caused by isoproterenol is not due to a direct block of Ca influx (7). Sugihara et al. (8) and Miyake et al. (9) prepared some tetrahydronaphthalene

derivatives and reported that the derivatives, AA497 and AA475, had β_2 -selective agonistic activities. The purpose of the present study is, therefore, to investigate the effects of butoxamine and propranolol on AA497 and AA475-induced and isoproterenol-induced Ca spike suppression and to relate the role of the β_2 -receptor to the suppression of Ca spikes.

Materials and Methods

Adult male guinea-pigs (Hartley strain, weighing 300–500 g) were stunned and bled. Strips of the taenia coli with some of the underlying circular muscle and myenteric plexus were dissected from the caecum.

Single sucrose-gap method: The method

used was essentially the same as that described by Bülbring and Tomita (10). A fine strip of the taenia, about 0.8 mm wide and 20–25 mm long, was inserted into a channel (1 mm diameter). A part of the tissue, 1.2 mm in length, was immersed in the test solution fluid (1 ml/min) at $36 \pm 1^\circ\text{C}$, while both ends were bathed in an isotonic sucrose solution. The sucrose solution was prepared by dissolving 92 g of sucrose in 1 liter of distilled water. The spontaneous spikes generated in the center part of the tissue were recorded across the sucrose gap with Ag-AgCl electrodes and measured by a spike counter (DSE322A, Dia-Medical). One end of the muscle was fixed to an acrylic rod, and the other end was attached by a thread to a mechano-electric transducer (SB-1T-H, Nihon Kohden) through a Bio-physiograph 180 system (Type 1206B) to record isometric tension changes. The tissue was mounted under a tension of 2.5 g. An oscillograph (Recti-Holy 8S, San-ei) was used for continuous recording of the electrical and mechanical responses.

Isotonic tension recording: Five mm strips of muscle were tied at both ends with silk threads; one thread was fixed to an arm of a vertical lever attached to a transducer (45071A, San-ei) for isotonic recording. Weights (1.5 g) were added to the other thread via a pulley. The strips were incubated in a jacketed organ bath (5 ml capacity) at 28°C .

A modified Krebs' solution of the following composition was used (mM): Na^+ , 135.7; K^+ , 5.9; Ca^{2+} , 2.5; Mg^{2+} , 1.2; Cl^- , 133.5; HCO_3^- , 15.5; glucose, 11.5; bubbled with 95% O_2 +5% CO_2 (pH 7.4–7.5). The solution was used only after previous equilibration for at least 30 min under the same conditions. To replace the NaCl with KCl, we prepared a solution containing 117.7 mM Na^+ and 24.0 mM K^+ , with the composition of the other ions unchanged. The experimental solutions

were modified by changing the calcium concentration to Ca-free solution with 0.5–1 mM EGTA. Locke-Ringer's solution of the following composition was partially used in tone recordings (mM): Na^+ , 160; K^+ , 5.6; Ca^{2+} , 2.2; Mg^{2+} , 2.1; Cl^- , 168.2; HCO_3^- , 6.0; glucose, 2.8.

AA497 (trans-5-hydroxymethyl-6-hydroxy-2-isopropylamino-1,2,3,4-tetrahydronaphthalene-1-ol)·HCl·3/2 H_2O , AA475 (trans-5-amino-6-hydroxy-2-isopropylamino-1,2,3,4-tetrahydronaphthalene-1-ol)·2HCl, butoxamine·HCl·1/2 H_2O , practolol (Takeda Chemical); dl-isoproterenol·HCl (Kaken-Kagaku); and EGTA (Nakarai) were used.

Results

Comparison of isoproterenol- and AA497-induced relaxations with and without butoxamine or practolol: The log dose-response curve of isoproterenol with and without practolol or butoxamine was compared with the curve of AA497. As shown in Figs. 1 and 2, the affinity (50% relaxing dose) of isoproterenol was 2 times greater than that of AA497. Practolol ($0.188 \mu\text{M}$) shifted the isoproterenol-induced relaxation curve more

Fig. 1. Cumulative log dose-relaxation curves of isoproterenol without (○) and with butoxamine (●, $0.16 \mu\text{M}$) or practolol (▲, $0.188 \mu\text{M}$). 100% relaxation means the maximal relaxing response induced by isoproterenol (32.3 nM). The values are presented as mean values ($N=5-6$) with S.E. Note that the isoproterenol-induced curve shifted to the right by practolol and hardly shifted any by butoxamine.

Fig. 2. Cumulative log dose-relaxation curve of AA497 without (○) and with butoxamine (●, 0.16 μ M) or practolol (▲, 0.188 μ M). 100% relaxation was the same as in Fig. 1. The values are presented as means (N=4-5) with S.E. Note that the AA497-induced curve shifted to the right to a lesser extent by practolol and to a greater extent by butoxamine than that in the case shown in Fig. 2.

than the AA497-induced one, whereas butoxamine (0.16 μ M) shifted non-competitively the AA497-induced curve and shifted very weakly the isoproterenol-induced one. Since AA497 has been reported as β_2 -selective (8), these results indicate that the β_2 -subtype of adrenergic β receptor is also in guinea pig taenia coli as well as the β_1 -subtype.

Effects of different concentrations of AA497 on spontaneous spike frequency and tension curves in 24 mM K^+ -depolarized solution: The decrease in isotonic tensions and suppression of spikes induced by various concentrations of AA497 were separately investigated. The relaxation was represented as a percent of maximally relaxed tones in the Ca^{2+} -free nutrient solution. The log dose-spike frequency curve and the log dose-tone curve induced by AA497 are shown in Fig. 3. In the 24 mM K^+ -nutrient solution, AA497-induced relaxation was accompanied by a transient cessation of spike frequency and a decrease in spike amplitude. Despite a lesser decrease in spike frequency, the log dose-tone curve induced by AA497 was still located in

Fig. 3. Relation between isotonic tension (dotted line) and spontaneous spike frequency (solid line) measured over a 3 min period, 3 min following the application of the 24 mM K^+ -Krebs' solution containing various concentrations of AA497. The spike frequency data are mean values (N=5) given as a percent of the total spike frequency measured over 3 min before applying the drug solution. The tension data (N=3) are expressed as a percent of the maximal relaxation in the Ca^{2+} -free solution. The bar indicates the S.E.

the lower concentration range. This phenomenon is very similar to that induced by isoproterenol (7). According to Fig. 3, the spike frequency increases again at high concentrations of AA497. The reasons for this are still unknown.

Spontaneous spike suppression by AA497 compared with that by calcium-free solution in 24 mM K^+ - or normal nutrient solution: In order to study the electropharmacological mechanism of AA497, the effect of AA497 on spontaneous spike frequency was compared with that of Ca^{2+} -free solution, both at normal and increased $[K^+]_0$. Spontaneous spike frequency-time curves of Ca^{2+} -free effects overlapped in normal and in 24 mM K^+ -depolarizing solution as shown in Fig. 4, indicating that Ca^{2+} -free effects on Ca^{2+} spikes were almost the same under both conditions. In contrast to the effects of the Ca^{2+} -free solution, the decrease in spike frequency induced by AA497 in 24 mM K^+ -depolarizing solution was much less than that in the normal nutrient solution (Fig. 5a and b). This phenomenon is also very similar to that induced by isoproterenol (7).

Fig. 4. Spontaneous spike frequency measured and time (min) elapsed after exchanging the normal K⁺-Krebs' solution with its Ca²⁺-free solution containing 1 mM EGTA (solid line) or exchanging the 24 mM K⁺-solution with its Ca²⁺-free solution containing 0.5 mM EGTA (dotted line). The data are mean values (N=3-5) given as a percent of values measured over 3 min before applying the test solution. The bars indicate the S.E.

The dose-spike frequency curve induced by isoproterenol and AA497 in the normal nutrient solution overlap in Fig. 6, although the log dose-tone curve for isoproterenol and AA497 as shown in Figs. 1 and 2 were

Fig. 5. a. Spontaneous spike frequency measured and time (min) elapsed after exchanging the normal Krebs' solution (solid lines) or the 24 mM K⁺-Krebs' solution (dotted lines) with the solution containing various AA497 concentrations (0.318, 3.18 and 31.8 μM). The data were obtained by the same procedure as that used in Fig. 4. The bars indicates the S.E. b. Typical recording of spontaneous spikes and corresponding isometric tensions. Upper: from 0 min to 9.5 min later after exchanging the normal solution with the solution containing 3.18 μM AA497. Lower: from 0 min to 10.5 min later after exchanging the 24 mM K⁺-depolarizing solution with the solution containing 31.8 μM AA497.

Fig. 6. Log dose-response curves of isoproterenol (Δ) and AA497 ($\circ$) for spontaneous spike frequency measured over 3 min, about 6 min following the application of the normal Krebs' solution containing various concentrations of isoproterenol or AA497. The data are mean values ($N=3-15$) given as a percent of values measured over 3 min before applying the test solution. The bars indicate the S.E.

Fig. 7. Spontaneous spike frequency measured and time (min) elapsed after exchanging the normal Krebs' solution with the solution containing $2.04 \mu\text{M}$ verapamil in the absence ($\circ$) and presence of $32 \mu\text{M}$ butoxamine ($\bullet$). The muscles were previously incubated with butoxamine for 3 min. The data ($N=5$) are represented in the same manner as in Fig. 4. The bars indicate the S.E.

separated (the former was 2-fold more potent than the latter). These results suggest that AA497 may be more closely related to Ca spike suppression than isoproterenol.

Effects of butoxamine and practolol on spontaneous spike suppression induced by AA497 and isoproterenol: We priorly confirmed that butoxamine had no effects on verapamil-induced spike suppression to determine the effect of the β_2 antagonist on AA497-induced spike suppression. The spontaneous spike suppression-time elapsed following the application of $2.04 \mu\text{M}$ verapamil in the presence of $32 \mu\text{M}$ butoxamine was compared with that in its absence (Fig. 7). Evidently butoxamine had no influence on the Ca spike suppression produced by verapamil. Butoxamine ($32 \mu\text{M}$) and practolol ($37.6-113 \mu\text{M}$) themselves had no effect on spontaneous spikes. These findings were essential for carrying out the following investigation. The spontaneous spike suppression-time curves after applying $1.21 \mu\text{M}$ isoproterenol were compared in the presence and absence of butoxamine (32 and $96 \mu\text{M}$) or practolol (37.6 and $113 \mu\text{M}$) as shown in Fig. 8. In the same way, the

Fig. 8. Spontaneous spike frequency measured and time (min) elapsed after exchanging the normal Krebs' solution with the solution containing $1.21 \mu\text{M}$ isoproterenol in the absence ($\circ$) and the presence of $37.6 \mu\text{M}$ and $113 \mu\text{M}$ practolol ($\blacktriangle$) or $32 \mu\text{M}$ and $96 \mu\text{M}$ butoxamine ($\bullet$). The muscles were previously incubated with practolol or butoxamine for 3 min. The data are mean values ($N=2-15$) given as a percent of values measured over 3 min before applying isoproterenol. The bars indicate the S.E.

effects of butoxamine ($32 \mu\text{M}$) and practolol ($37.6 \mu\text{M}$) on AA497 ($3.18 \mu\text{M}$)-induced or AA475 ($3.23 \mu\text{M}$)-induced suppression of spontaneous spikes are shown in Figs. 9 and 10. Butoxamine ($32 \mu\text{M}$, β_2 -selective antagonist) could inhibit particularly the AA497 (β_2 -selective agonist)-induced effect

Fig. 9. Spontaneous spike frequency measured and time (min) elapsed after exchanging the normal Krebs' solution with the solution containing $3.18 \mu\text{M}$ AA497 in the absence ($\circ$) and the presence of $37.6 \mu\text{M}$ practolol ($\blacktriangle$) or $32 \mu\text{M}$ butoxamine ($\bullet$). The muscles were previously incubated with practolol or butoxamine for 3 min. The data are mean values ($N=2-6$) and are represented in the same manner as that in Fig. 7. The bars indicate the S.E.

Fig. 10. Spontaneous spike frequency measured and time (min) elapsed after exchanging the normal Krebs' solution with the solution containing $3.23 \mu\text{M}$ AA475 in the absence ($\circ$) and the presence of $37.6 \mu\text{M}$ practolol ($\blacktriangle$) or $32 \mu\text{M}$ butoxamine ($\bullet$). The muscles were previously incubated with practolol or butoxamine for 3 min. The data are mean values ($N=4-9$) and are represented in the same manner as that in Fig. 7. The bars indicate the S.E.

to a greater extent. In contrast to the effects of butoxamine, almost the same concentration ($37.6 \mu\text{M}$) of practolol could hardly inhibit the AA497, AA475 and isoproterenol induced-suppressions of spontaneous spikes. Since the potency order of the β_2 -selectivity was AA497>AA475>isoproterenol (8, 9),

these results demonstrate that in the adrenergic β -receptor-mediated process in taenia coli, β_2 -mechanisms are more closely related to the Ca spike suppression than the β_1 -mechanisms are.

Discussion

The early electric current in the taenia coli of guinea pig is not carried principally by Na^+ , but by an influx of Ca^{2+} (11-13). Isoproterenol was reported to block spike activity with little or no change in membrane conductance (10, 14) and to cause a small hyperpolarization of the membrane (15). Casteels and Raeymaekers (16) reported that the intracellular Ca stores in taenia coli were sensitive to beta agonists and not influenced by depolarization of the outer membrane. In the previous paper (7), we observed that the suppression of the spike frequency induced by a low concentration of isoproterenol tended to be transient, despite sustained relaxation. Also, we confirmed that in the presence of 24 mM K^+ , beta receptor activation led to relaxation by an effect not involving the electrical activity of the membrane, because despite the sustained relaxation, spontaneous spike suppression induced by isoproterenol lessened in the 24 mM K^+ -depolarizing solution rather than in a normal nutrient solution. In the present study, AA497 (a β_2 -selective agonist)-induced suppression of the spontaneous spikes in 24 mM K^+ -depolarizing solution showed the same characteristics as our previous results. Here we obtained again the same conclusion that spontaneous spike suppression induced by beta agonist in the normal solution may be caused by a secondary (not direct) reaction such as increase in Ca uptake into the Ca stores and so on. As clearly shown in Figs. 8-10, $37.6 \mu\text{M}$ practolol could not inhibit β -agonist-induced spontaneous spike suppression, but $32 \mu\text{M}$ butoxamine could completely do so, par-

ticularly the AA497 (3.18 μ M)-induced spontaneous spike suppression. As the β_2 -selectivity of an adrenergic agonist such as AA475 became less, butoxamine seemed to inhibit it to a lesser extent. This suggests that adrenergic β_2 -mechanisms may be more closely related to Ca spike-generating mechanisms.

Based on the inhibitory effects of catecholamines on spontaneous contraction in rabbit jejunum, Lands et al. (1, 2) postulated that the small intestine generally belongs to the β_1 -receptor type. Our data also show that the isoproterenol-induced relaxation in guinea pig taenic coli is produced by β_1 -adrenergic mechanisms as shown in Fig. 1. It was, however, considered that all the catecholamine-induced responses in guinea-pig taenia coli were not necessarily caused by only β_1 -adrenergic mechanisms. The present study indicates that the catecholamine-induced Ca spike suppression is mediated by β_2 -adrenergic rather than by β_1 -adrenergic mechanisms. Obviously, catecholamine-induced relaxation was entirely distinct from catecholamine-induced suppression of Ca spikes since the former is attributed to β_1 -mechanisms and the latter to β_2 -mechanisms.

Acknowledgement: We would like to thank Mr. H. Hashimoto for competent technical assistance, and Dr. M. Nishikawa (Takeda Chemical Industries) for kindly providing the AA479 and AA475.

References

- 1) Lands, A.M., Arnold, A., McAuliff, J.P., Luduena, F.P. and Brown, T.G., Jr.: Differentiation of receptor systems activated by sympathomimetic amines. *Nature* **214**, 597–598 (1967)
- 2) Lands, A.M., Luduena, F.P. and Buzzo, H.J.: Differentiation of receptors responsive to isoproterenol. *Life Sci.* **6**, 2241–2249 (1967)
- 3) Minneman, K.P. and Molinoff, P.B.: Classification and quantitation of β -adrenergic receptor subtypes. *Biochem. Pharmacol.* **29**, 1317–1323 (1980)
- 4) O'Donnell, S.R. and Wanstall, J.C.: The importance of choice of agonist in studies designed to predict $\beta_2 : \beta_1$ adrenoceptor selectivity of antagonists from pA_2 values on guinea-pig taenia and atria. *Naunyn Schmiedebergs Arch. Pharmacol.* **308**, 183–190 (1979)
- 5) Minneman, K.P., Hegstrand, L.R. and Molinoff, P.B.: Simultaneous determination beta-1 and beta-2 adrenergic receptors in tissues containing both receptor subtypes. *Mol. Pharmacol.* **16**, 34–46 (1979)
- 6) Cohen, M.L. and Wiley, K.S.: Beta₁ and beta₂ receptor mechanism in rat jugular veins: Differences between norepinephrine and isoproterenol-induced relaxation. *Life Sci.* **23**, 1997–2006 (1978)
- 7) Kimura, I., Kimura, M. and Yoshimura, Y.: Quantitization of Ca spike frequency in a single sucrose gap and mechanical relaxation induced by Ca antagonist, isoproterenol and papaverine. *Arch. Int. Pharmacodyn. Ther.* **252**, 40–52 (1981)
- 8) Sugihara, H., Ukawa, K., Kuriki, H., Nishikawa, M. and Sanno, Y.: Syntheses and β -adrenoceptor activities of 2-alkylamino-6-hydroxy-5-hydroxymethyl-1,2,3,4-tetrahydro-1-naphthalenols. *Chem. Pharm. Bull. (Tokyo)* **25**, 2988–3002 (1977)
- 9) Miyake, A., Kuriki, H., Toda, N., Nishikawa, M. and Oka, Y.: The synthesis of 2,5-diamino-6-hydroxy-1,2,3,4-tetrahydro-1-naphthalenol derivatives. *Chem. Pharm. Bull. (Tokyo)* **25**, 3066–3074 (1977)
- 10) Bülbring, E. and Tomita, T.: Suppression of spontaneous spike generation by catecholamines in the smooth muscles of the guinea-pig taenia coli. *Proc. R. Soc. Lond. [Biol.]* **172**, 103–119 (1969)
- 11) Bülbring, E. and Tomita, T.: Effect of calcium, barium and manganese on the action of adrenaline in the smooth muscle of the guinea-pig taenia coli. *Proc. R. Soc. Lond. [Biol.]* **172**, 121–136 (1969)
- 12) Kuriyama, H. and Tomita, T.: The action potential in the smooth muscle of the guinea pig taenia coli and ureter studied by the double sucrose gap method. *J. Gen. Physiol.* **55**, 147–162 (1970)
- 13) Inomata, H. and Kao, C.Y.: Ionic currents in the guinea-pig taenia coli. *J. Physiol. (Lond.)* **255**, 347–378 (1976)
- 14) Jager, L.P.: The effect of catecholamines and ATP on the smooth muscle cell membrane of the guinea-pig taenia coli. *Eur. J. Pharmacol.* **25**, 372–382 (1974)
- 15) Bülbring, E. and den Hertog, A.: The action of

isoprenaline on the smooth muscle of the guinea-pig taenia coli. *J. Physiol. (Lond.)* **304**, 277–296 (1980)

16) Casteels, R. and Raeymaekers, L.: The action

of acetylcholine and catecholamines on an intracellular calcium store in the smooth muscle cells of the guinea-pig taenia coli. *J. Physiol. (Lond.)* **294**, 51–68 (1979)