J. Pharm. Pharmacol. 1987, 39: 439–443
Received October 15, 1986

Ca^{2+} channel-blocking effect of the yohimbine derivatives, 14β-benzoyloxyyohimbine and 14β-*p*-nitrobenzoyloxyyohimbine

K. WATANABE, S. YANO*, H. HORIUCHI, E. YAMANAKA†, N. AIMI†, S. SAKAI†

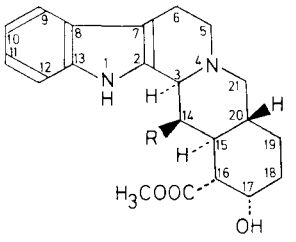
Laboratory of Chemical Pharmacology, Department of Drug Evaluation and Toxicological Sciences and †Laboratory of Pharmaceutical Chemistry, Department of Medicinal Materials, Faculty of Pharmaceutical Sciences, Chiba University, 1–33 Yayoi-cho, Chiba 260, Japan

Yohimbine possesses a relatively selective α_2-adrenoceptor antagonistic effect and a weak Ca^{2+} channel blocking activity, so two derivatives, 14β-benzoyloxyyohimbine and 14β-*p*-nitrobenzoyloxyyohimbine were compared with yohimbine using those parameters. The two derivatives blocked Ca^{2+}-induced contraction of the rat perfused mesenteric vascular bed at 3×10^{-6} M, and developed dose-dependent shifts of the dose-response curve to the right in concentrations from 3×10^{-6} to 10^{-5} M. Yohimbine showed a weak Ca^{2+} channel-blocking effect only at a concentration of 10^{-5} M. On the other hand, both derivatives at 3×10^{-6} M did not affect the dose-response curve to noradrenaline-induced vasoconstriction. Furthermore, at 10^{-8} M they had no effect on the dose-response curve to clonidine-induced inhibition of the twitch response of the rat isolated vas deferens evoked by electric stimulation, whereas yohimbine significantly attenuated clonidine-induced inhibition. These results indicate that introduction of bulky substituents into the 14-position of yohimbine intensely potentiated the Ca^{2+} channel-blocking effects and reduced α-adrenoceptor antagonistic effects compared with those of yohimbine, and suggest that chemical modifications of yohimbine may lead to structurally new types of Ca^{2+} channel-blockers.

Yohimbine and its diastereomers are widely used as pharmacological tools for investigating α-adrenoceptors (Lambert et al 1978; McGrath 1982; Goldberg & Robertson 1983). They are also known to have weak Ca^{2+} channel-blocking effects as well as unidentified antagonistic effects on agonist-induced contractile responses of the rat aorta (Godfraind et al 1982, 1983).

We have previously reported on the pharmacological effects of a number of indole alkaloids (Harada et al 1974, 1979; Ozaki et al 1980; Ozaki & Harada 1981, 1982, 1983; Watanabe et al 1985) and found that hirsutine, an indole alkaloid in *Uncaria rhynchophylla* Miq (Haginiwa et al 1973), had a ganglion-blocking effect (Harada et al 1979; Ozaki et al 1980; Ozaki & Harada 1982, 1983) and a Ca^{2+} channel-blocking effect (Ozaki & Harada 1981).

In the present study, we have investigated the pharmacological properties of the yohimbine derivatives, 14β-benzoyloxyyohimbine (I) and 14β-*p*-nitrobenzoyloxyyohimbine (II) (Yamanaka et al 1983, 1984), for their Ca^{2+} channel-blocking effects and α-adrenoceptor antagonistic effects.



Yohimbine	R = H-
I	R = C_6H_5COO-
II	R = *p*-$NO_2C_6H_4COO$-

METHODS

Rat isolated perfused superior mesenteric vascular bed

Male Wistar rats, 240–310 g, were anaesthetized with sodium pentobarbitone (50 mg kg^{-1} i.p.). The perfused mesenteric vascular bed was prepared using a modification of the method of McGregor (1965). The abdomen was opened and the pancreatico-duodenal, ileo-colic and colic branches of the artery were tied. The superior mesenteric artery was cannulated at its origin distally from the aorta and perfusate was delivered at a low flow rate to remove the blood in the vascular bed. Then, the mesenteric

* Correspondence.

vascular bed was separated from the intestine. During the perfusion, the isolated mesenteric vascular bed was placed in a water-jacketed organ bath maintained at 37 °C and covered with absorbent gauze moistened with perfusate to prevent drying. Modified Krebs solution (mM): NaCl 118·07, $NaHCO_3$ 25·00, glucose 11·10, KCl 4·69, $CaCl_2$ 2·58, NaH_2PO_4 1·15, $MgCl_2$ 1·18, EDTA-Na_2 0·03 was used as perfusate, which was gassed with 95% O_2/5% CO_2, and delivered at a constant flow rate of 4·5 mL min^{-1} by a roller pump (Tokyo Kagaku Sangyo, Ltd, TMP-10H). Changes in the perfusion pressure were measured with a biophysiograph (San-ei, 180SYSTEM) via a pressure transducer (Toyo Baldwin Co., Ltd, MPU-0·5-290-0-3) and amplified with an amplifier (Nihon Kohden, AD2-22), then recorded on an ink writing oscillograph (Nihon Kohden, WI-130). Vasoconstrictors were injected in a constant volume of 0·1 mL into the perfusate via pressure-resistant rubber proximal to the tissue.

All tissues were allowed to equilibrate for 30 min before initiation of experiments.

The effects on $CaCl_2$ (Ca^{2+}). After allowing the tissue to be equilibrated with modified Krebs solution, KCl (4 M in a volume of 0·1 mL) was injected at 5 min intervals to obtain the constant pressor responses of the tissue. The tissue was then washed with Ca^{2+}-free, modified Krebs solution ($CaCl_2$ was omitted and 0·1 mM EGTA was added). After KCl-induced pressor responses had disappeared, the tissue was perfused with Ca^{2+}-free, high K^+ modified Krebs solution ($CaCl_2$ omitted, NaCl replaced with the same molar strength of KCl) and dose-response curves for Ca^{2+} injection (3×10^{-4}–10^{-1} or 1 M in a volume of 0·1 mL) in the absence or presence of the test drug were obtained.

The effects on noradrenaline (NA). After NA (10^{-3}–3×10^{-3} M in a volume of 0·1 mL) was injected at 5 min intervals to obtain the constant pressor responses of the tissue, dose-response curves were obtained to NA (3×10^{-6}–10^{-2} or 3×10^{-2} M in a volume of 0·1 mL) in the absence or presence of the test drug.

Vasoconstrictor responses were measured as increases in perfusion pressure above basal perfusion pressure and converted to a percentage of the maximal responses in control.

Rat isolated vas deferens

The effects on clonidine. Male Wistar rats, 250–320 g, were stunned and exsanguinated. The vas deferens was dissected from the surrounding tissues, and the prostatic portion (approximately 2 cm) used. The preparation was suspended in a water-jacketed organ bath (20 mL) containing Krebs-Henseleit solution (mM): NaCl 112·08, $NaHCO_3$ 25·00, glucose 11·49, KCl 5·90, $CaCl_2$ 1·97, NaH_2PO_4 1·22, $MgCl_2$ 1·18, EDTA-Na_2 0·03, was gassed with 95% O_2/5% CO_2 maintained at 37 °C, and a resting tension of 0·3 g was applied.

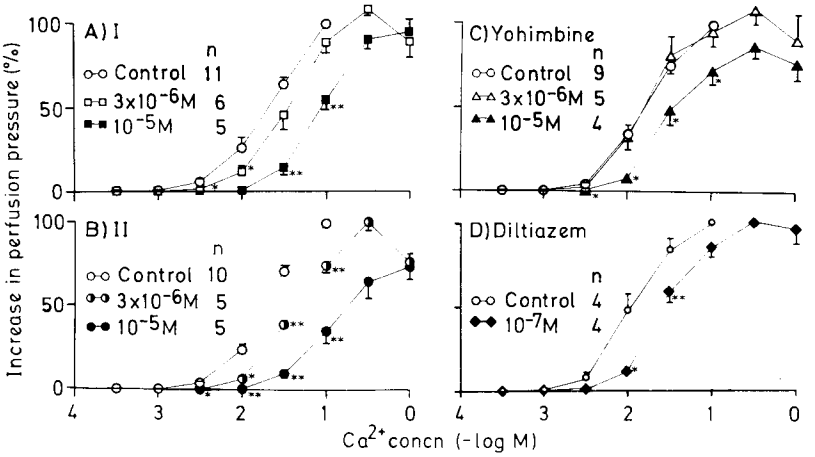


FIG. 1. Effects of 14β-benzoyloxyyohimbine (A), 14β-*p*-nitrobenzoyloxyyohimbine (B), yohimbine (C) and diltiazem (D) on the concentration-response curves to Ca^{2+}-induced contractile response of the rat isolated perfused mesenteric vascular bed. In A, B and C, the control-response curves were plotted from summed data of the individual controls of the drug groups. Each value represents the mean ± s.e. $^*P < 0·05$, $^{**}P < 0·01$, compared with control response by the paired *t*-test.

After allowing the tissue to be equilibrated for 60 min, the tissue was transmurally stimulated (5 Hz, 0·5 ms duration, supramaximal voltage of 50 V) with platinum ring-needle electrodes for 2 s, at 5 min intervals. Transmural stimulation was made by an electronic stimulator (Nihon Kohden, MSE-3R) coupled to an isolating unit (Nihon Kohden, MSE-JH) and controlled by an autotimer (Shin-ei Co., TM-101). Twitch responses were measured isometrically with a biophysiograph via a transducer (Toyo Baldwin Co., Ltd, T7-8-240) and recorded on a recorder (Hitachi, 056).

After constant responses to stimulation had been obtained, clonidine was added cumulatively and a dose-response curve for the inhibitory effect on the twitch response was obtained (as soon as the responses to the previous dose of clonidine became constant, the next dose was added). The tissue was then washed, and a test drug added to the bath. After allowing the tissues to equilibrate for 30 min, the second dose-response curve to clonidine was obtained.

Drugs used were: (−)-noradrenaline bitartrate (Wako Pure Chemical Industries, Ltd); calcium chloride (Wako Pure Chemical Industries, Ltd); clonidine hydrochloride (donated by Sankyo Co, Ltd); yohimbine hydrochloride (Yoh; Wako Pure Chemical Industries, Ltd); diltiazem hydrochloride (donated by Tanabe Seiyaku Co., Ltd); 14β-benzoyloxyyohimbine hydrochloride (I) and 14β-*p*-nitrobenzoyloxyyohimbine (II) were synthetized in our laboratory. NA was dissolved in 10^{-4} M ascorbic acid (Wako Pure Chemical Industries, Ltd). Ca^{2+}, clonidine, Yohimbine, diltiazem and I were dissolved in distilled water, and II was dissolved in aqueous HCl 10^{-3} M.

Results were statistically analysed using the paired *t*-test. *P* values at 0·05 were considered to be significant.

RESULTS

Rat isolated perfused superior mesenteric vascular bed

Pressor responses were reproducible over the experimental periods. Neither bolus injection of vehicles (in a volume of 0·1 mL) nor the perfusion of test drugs affected the basal pressure values.

Effects on Ca^{2+}-induced vasoconstriction. I (3×10^{-6} and 10^{-5} M) dose-dependently caused significant shifts of the dose-response curve to Ca^{2+}-induced vasoconstriction to the right, indicating the Ca^{2+} channel-blocking effect of I (Figs 1A, 2A). II (3×10^{-6} and 10^{-5} M) also shifted the dose-response curve to the right and the potency was somewhat stronger than that of I (Figs 1B, 2A). On the other hand yohimbine significantly affected the dose-response curve at 10^{-5} M but not at 3×10^{-6} M (Fig. 1C). Diltiazem, a typical Ca^{2+} channel-blocker of benzothiazepine type, at 10^{-7} M, showed a significant Ca^{2+} channel-blocking effect (Fig. 1D).

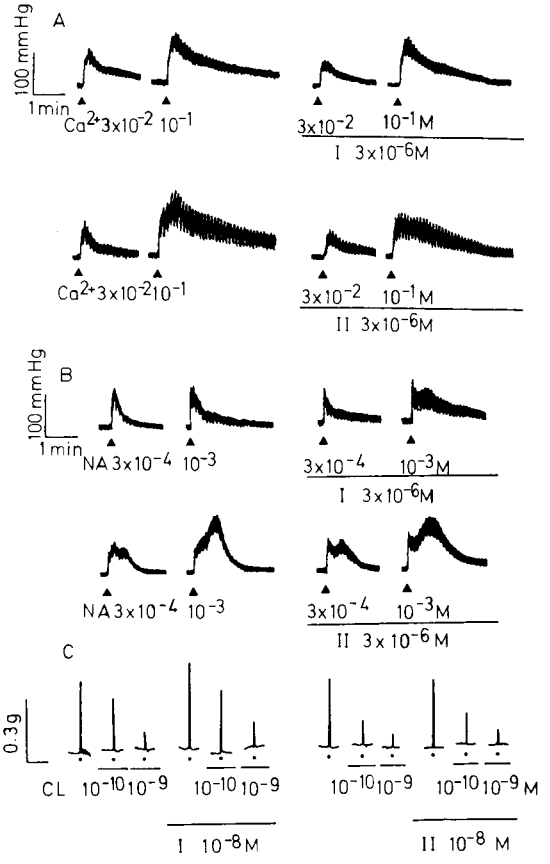


FIG. 2. Records of the effects of 14β-benzoyloxyyohimbine (I) and (II) 14β-*p*-nitrobenzoyloxyyohimbine and on Ca^{2+}-induced (A) or NA-induced (B) contractile responses of the rat isolated perfused mesenteric vascular bed, and on clonidine (CL)-induced (C) inhibition of the twitch response of the rat isolated vas deferens evoked by electric stimulation (5 Hz, 0·5 ms, 50 V; indicated with ·).

Effects of NA-induced vasoconstriction. Both I and II in a concentration of 3×10^{-6} M did not affect the dose-response curve to NA-induced vasoconstriction, while yohimbine in the same concentration significantly shifted the dose-response curve to the right (Figs 3, 2B).

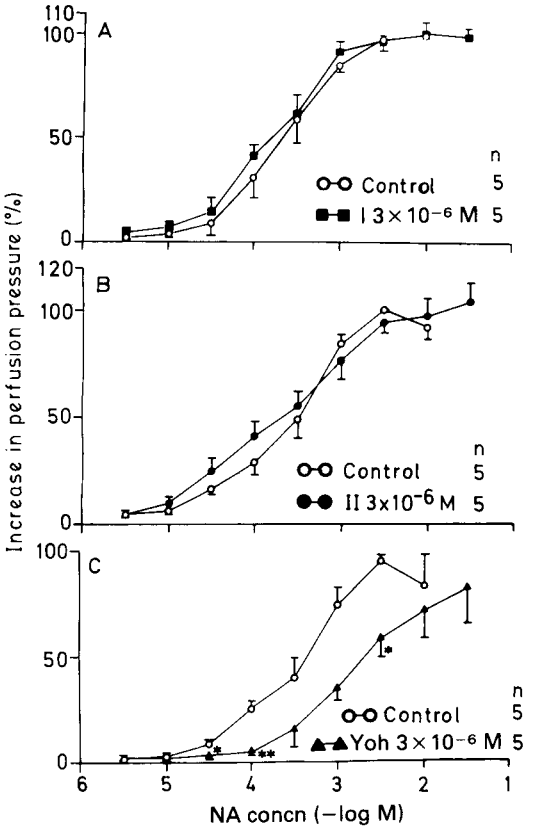


FIG. 3. Effects of 14β-benzoyloxyyohimbine (A), 14β-*p*-nitrobenzoyloxyyohimbine (B) and yohimbine (C) on the concentration-response curves to NA-induced contractile response of the rat isolated perfused mesenteric vascular bed. Each value represents the mean ± s.e. $*P < 0.05$, $**P < 0.01$, compared with the control response by the paired *t*-test.

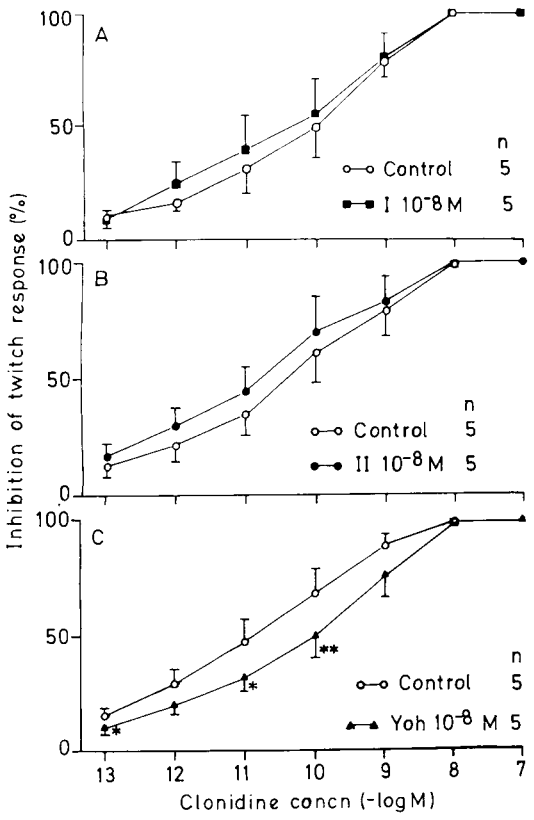


FIG. 4. Effects of 14β-benzoyloxyyohimbine (A), 14β-*p*-nitrobenzoyloxyyohimbine (B) and yohimbine (C) on the concentration-response curves to clonidine-induced inhibition of the twitch response of the rat isolated vas deferens evoked by electric stimulation (5 Hz, 0·5 ms, 50 V). Each value represents the mean ± s.e. $*P < 0.05$, $**P < 0.01$, compared with the control response by the paired *t*-test.

Rat isolated vas deferens

Effect on clonidine-induced inhibition of the twitch response. Both I and II at a concentration of 10^{-8} M did not affect the dose-response curve to clonidine-induced inhibition of the twitch response, while yohimbine in the same concentration significantly shifted the dose-response curve to the right (Figs 4, 2C).

DISCUSSION

In the present study, we investigated α-adrenoceptor antagonistic effects and Ca^{2+} channel-blocking effects of yohimbine-related compounds which were provided with the β-orientated substituent at the 14-position of yohimbine.

In the K^+-depolarized mesenteric vascular bed preparation of the rat, Ca^{2+}-induced contractile responses are thought to result from influx of extracellular Ca^{2+} which passes through potential-dependent Ca^{2+} channels. On the other hand, NA-induced constriction of the preparation is thought to be caused by the stimulation of α_1-adrenoceptors because the postsynaptic α-adrenoceptors of the rat mesenteric artery are predominantly α_1-adrenoceptors. We did not observe any change in basal perfusion pressure by clonidine in concentrations up to 10^{-3} M (data not shown). On the other hand, the twitch response of rat isolated vas deferens induced by electric stimulation is caused by the stimulation of sympathetic nerves, but not by direct stimulation of the smooth muscles, because the response is abolished by pretreatment with 10^{-6} M tetrodotoxin. The inhibition of the response by clonidine is thought to result from the stimulation of the presynaptic α_2-adrenoceptors. Thus, the present results indicate

that I and II possess a Ca^{2+} channel-blocking effect which is much stronger than that of yohimbine, with only a small α-adrenoceptor antagonistic effect, unlike yohimbine.

A number of investigators have studied structure-activity relationships for yohimbine and its related compounds concerning α_1- and α_2-adrenoceptors (Lambert et al 1978; McGrath 1982; Goldberg & Robertson 1983; Ferry et al 1983; Baldwin et al 1985; Watanabe et al 1985). The generally accepted points are that the indole structure, the nitrogen atom at the 4-position and the methoxycarbonyl group at the 16-position of yohimbine constitute its binding sites to α-adrenoceptors, while planarity of the A, B, C and D rings of yohimbine determines the affinity to the receptors (Ferry et al 1983; Baldwin et al 1985). We have previously found that (−)-indoloquinolizidine, which consists of the A, B, C and D rings in yohimbine, possesses both α_1- and α_2-adrenoceptor antagonistic effects; we therefore concluded that A, B, C and D rings were the minimum requirement for α-adrenoceptor antagonistic activity (Watanabe et al 1985).

Introduction of a β-orientated benzoyloxy group to the 14-position of yohimbine results in a loss of planarity of the A, B, C and D rings and thereby α-adrenoceptor antagonistic effects of the compounds are thought to be reduced compared with those of yohimbine. Furthermore, introduction of the benzoyloxy group to the 14-position causes potentiation of Ca^{2+} channel-blocking activity while replacement of the benzoyloxy group by a *P*-nitrobenzoyloxy group seems to potentiate the activity.

NA causes hydrolysis of phosphatidylinositol-4,5-bisphosphate to generate inositoltrisphosphate and diacylglycerol (PI response) (Nishizuka 1984), and inositoltrisphosphate in turn induces the release of Ca^{2+} from the endoplasmic reticulum (Streb et al 1983; Berridge & Irvine 1984). Apart from such events, some Ca^{2+} will be mobilized through α-adrenoceptor operated Ca^{2+} channels by unknown mechanisms. Thus, the NA-induced contractile response of rat isolated mesenteric vascular bed is thought to be caused by Ca^{2+} via the Ca^{2+}-calmodulin system (Hartshorne & Siemankowski 1981) and/or protein kinase C system (Forder et al 1985). In this study, however, I and II did not affect NA-induced vasoconstriction, thus suggesting an inability of these compounds to attenuate agonist-induced vasoconstriction.

In conclusion, I and II were found to possess Ca^{2+} channel-blocking activity virtually without α-adrenoceptor antagonistic effects. Their chemical structures are quite different from those of currently available Ca^{2+} channel-blockers. Therefore, it might be expected that further chemical modifications should lead to a new type of Ca^{2+} channel-blocker.

REFERENCES

Baldwin, J. J., Huff, J. R., Randall, W. C., Vacca, J. P., Zrada, M. M. (1985) Eur. J. Med. Chem. 20: 67–69

Berridge, M. J., Irvine, R. F. (1984) Nature 312: 315–321

Ferry, N., Goodhardt, M., Hanoune, J., Sevenet, T. (1983) Br. J. Pharmacol. 78: 359–364

Forder, J., Scriabine, A., Rasmussen, H. (1985) J. Pharmacol. Exp. Ther. 253: 267–273

Godfraind, T., Miller, R. C., Lima, J. S. (1982) Arch. Int. Pharmacodyn. 260: 280–281

Godfraind, T., Miller, R. C., Lima, J. S. (1983) Br. J. Pharmacol. 80: 115–121

Goldberg, M. R., Robertson, D. (1983) Pharmacol. Rev. 35: 143–180

Haginiwa, J., Sakai, S., Aimi, N., Yamanaka, E., Shinma, N. (1973) Yakugaku Zasshi 93: 448–452

Harada, M., Ozaki, Y., Sato, M. (1974) Chem. Pharm. Bull. 22: 1372–1377

Harada, M., Ozaki, Y., Ohno, H. (1979) Ibid. 27: 1069–1074

Hartshorne, D. J., Siemankowski, R. F. (1981) Ann. Rev. Physiol. 43: 519–530

Lambert, G. A., Lang, W. J., Friedman, E., Meller, E., Gershon, S. (1978) Eur. J. Pharmacol. 49: 39–48

McGrath, J. C. (1982) Biochem. Pharmacol. 31: 467–484

McGregor, D. D. (1965) J. Physiol. (London) 177: 21–30

Nishizuka, Y. (1984) Nature 308: 693–698

Ozaki, Y., Harada, M. (1981) Eighth International Congress of Pharmacology, Tokyo, 796p

Ozaki, Y., Harada, M. (1982) Jap. J. Pharmacol. 32: Suppl. 189p

Ozaki, Y., Harada, M. (1983) Ibid. 33: 463–471

Ozaki, Y., Harada, M., Sakai, S. (1980) Ibid. 30: Suppl. 137p

Streb, H., Irvine, R. F., Berridge, M. J., Schulz, I. (1983) Nature 306: 67–69

Watanabe, K., Yano, S., Ito, Y. (1985) Jap. J. Pharmacol. 39: Suppl. 223p

Yamanaka, E., Murata, E., Kasamatsu, S., Aimi, N., Sakai, S., Ponglux, D., Wongseripipa, S., Supavita, T. (1983) Tetrahedron Lett. 24: 3861–3864

Yamanaka, E., Ono, M., Kasamatsu, S., Aimi, N., Sakai, S. (1984) Chem. Pharm. Bull. 32: 818–821