Biochemical Pharmacology, Vol. 36, No. 5, pp. 609–612, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00
Pergamon Journals Ltd.

EFFECTS OF DJ-7141, A NEW α_2-ADRENOCEPTOR AGONIST, ON CATECHOLAMINE SECRETION FROM ISOLATED BOVINE ADRENAL MEDULLARY CELLS

MOTOO OKA, MIR MISBAHUDDIN, YASUKO ISHIMURA and KYOJI MORITA

Department of Pharmacology, Tokushima University School of Medicine, 3-Kuramoto, Tokushima 770, Japan

(*Received* 1 *July* 1986; *accepted* 1 *August* 1986)

Abstract—The effects of a newly synthesized α_2-adrenoceptor agonist (an imidazole derivative, DJ-7141) on catecholamine secretion from isolated bovine adrenal medullary cells were examined. DJ-7141 did not affect basal catecholamine secretion, but inhibited catecholamine secretion induced by stimulation of the nicotinic ACh receptor. This inhibitory effect of DJ-7141 was less than that of clonidine, another α_2-agonist. DJ-7141 also inhibited $[^{45}Ca]^{2+}$ uptake by the cells induced by nicotinic stimulation. DJ-7141 did not affect catecholamine secretion induced by high K^+ concentration. Its inhibitory effect on nicotine-induced catecholamine secretion was not restored by increase in either the nicotine or Ca^{2+} concentration of the medium, suggesting that it interfered with the coupling between nicotinic ACh receptor stimulation and Ca^{2+}-channel activation. The inhibitory effect of DJ-7141 seemed to be independent of its effect on α_2-adrenoceptors, because its effect was not antagonized by the α_2-adrenoceptor antagonists yohimbine and DG-5128, which both had no effect on either basal or nicotine-induced catecholamine secretion.

A newly synthesized α_2-adrenoceptor agonist (an imidazole derivative, DJ-7141, Fig. 1) has little effect on central α_2-adrenoceptors, and therefore its hypotensive effect is less than that of another well-known α_2-agonist, clonidine [1]. However, this new α_2-agonist acts selectively on pre-synaptic α_2-adrenoceptors on sympathetic nerve terminals and thereby inhibits smooth muscle contraction caused by electrical stimulation [2].

Recently, we found that DJ-7141 reduced the plasma catecholamine level in dogs, although its effect was less than that of clonidine (unpublished observations). This effect of DJ-7141 seemed to be due to its stimulatory effect on pre-synaptic α_2-adrenoceptors, resulting in inhibition of catecholamine release from sympathetic nerve terminals.

In this work, using isolated bovine adrenal medullary cells we examined whether DJ-7141 also inhibited catecholamine secretion from adrenal medulla. Results showed that this new α_2-agonist inhibited catecholamine secretion induced by nicotinic ACh receptor stimulation, but not that induced by high K^+. Its mode of action was also examined.

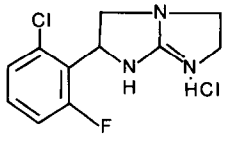

Fig. 1. Chemical structure of DJ-7141 {2-(2-chloro-6-fluorophenyl)-2,3,5,6-tetrahydro-1*H*-imidazo [1,2-*a*] imidazole hydrochloride}.

MATERIALS AND METHODS

Isolated bovine adrenal medullary cells were prepared by sequential digestion of adrenal medullary slices with 0.05% collagenase as described previously [3]. The isolated cells were finally suspended in Krebs–Ringer phosphate (KRP) buffer solution (154 mM NaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 1.1 mM $MgSO_4$, 0.85 mM NaH_2PO_4, 2.15 mM Na_2HPO_4, 10 mM glucose and 0.5% bovine serum albumin, pH 7.4) at about 4×10^6 cells/ml. The cell suspension (final volume 2 ml) was pre-incubated at 37° in the absence or presence of drugs (DJ-7141, clonidine, yohimbine or DG-5128) for 5 min and then stimulated with nicotine (10 μM) or high K^+ (56 mM) for 2 min. In the 56 mM K^+ solution, NaCl was replaced by KCl on an equimolar basis. The reaction was terminated by transferring the reaction mixtures to an ice–water bath and the cells were preincubated by centrifugation at 600 *g* for 5 min. Catecholamine in the cells and medium was extracted with 0.4 N perchloric acid and measured by a modification of the trihydroxyindole method [4]. For measurement of $[^{45}Ca]^{2+}$ uptake by the cells [5], 3.0 μCi of $[^{45}Ca]^{2+}$ was added to the incubation medium. After incubation, the tubes were rapidly chilled in ice and when the cells had been stimulated with nicotine, hexamethonium (3×10^{-4} M) was added to stop the reaction. The cells were washed three times with ice-cold Ca^{2+}-free KRP buffer containing 0.5% bovine serum albumin. The $[^{45}Ca]^{2+}$ in the cells was extracted with 0.4 N perchloric acid and measured in a liquid scintillation counter.

DJ-7141 was synthesized and donated by Daiichi Seiyaku Co. (Tokyo, Japan). The following drugs

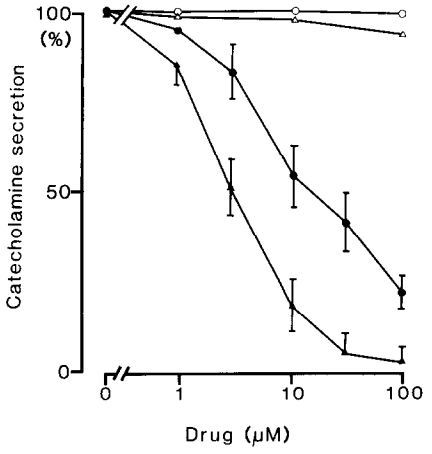

Fig. 2. Inhibitory effects of DJ-7141 and clonidine on catecholamine secretion from isolated bovine adrenal medullary cells induced by nicotine and high K^+. Cells were incubated with or without various concentrations of DJ-7141 or clonidine and stimulated with nicotine (10 μM) or high K^+ (56 mM) as described in Materials and Methods. Catecholamine secretion induced by nicotine or high K^+ was ~6–7% of the total cellular catecholamine content. The maximal secretion induced by each stimulant was expressed as 100%. Values for nicotine-induced catecholamine secretion in the presence of DJ-7141 (●) or clonidine (▲), and high K^+-induced catecholamine secretion in the presence of DJ-7141 (○) or clonidine (△) are means for three or four experiments; standard deviations are shown by vertical bars.

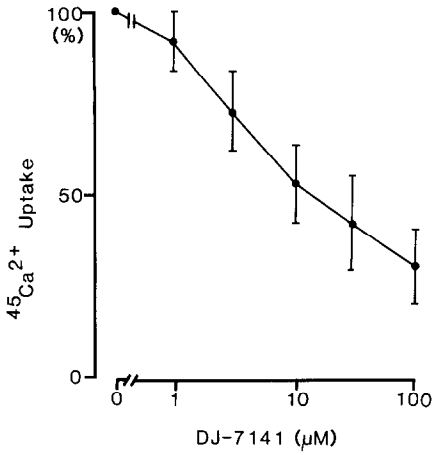

Fig. 3. Inhibitory effect of DJ-7141 on nicotine-induced $[^{45}Ca]^{2+}$ uptake by isolated bovine adrenal medullary cells. Cells were incubated with $^{45}CaCl_2$ as described in Materials and Methods. $[^{45}Ca]^{2+}$ taken up by the cells induced by nicotine was 58 ± 8 nmol $Ca^{2+}/4 \times 10^6$ cells and was expressed as 100%. Points and bars show nicotine-induced $[^{45}Ca]^{2+}$ uptake by the cells in the presence of various concentrations of DJ-7141 as means and standard deviations of three or four experiments.

were used: clonidine hydrochloride (Tokyo Kasei), yohimbine hydrochloride (Sigma), DG-5128 (Daiichi Seiyaku) and nicotine (Sigma).

RESULTS

Isolated bovine adrenal medullary cells secrete catecholamine on stimulation of the nicotinic ACh receptor, but not the muscarinic ACh receptor [5]. Therefore, in this study, nicotine was used as a stimulant. High K^+ (56 mM) was also used to initiate secretion of catecholamine. Figure 2 shows the effects of different concentrations of DJ-7141 on catecholamine secretion from isolated bovine adrenal medullary cells induced by nicotine (10 μM) or high K^+ (56 mM). The effects of different concentrations of clonidine are shown for comparison. DJ-7141 alone did not affect basal catecholamine secretion, but caused concentration-dependent inhibition of catecholamine secretion induced by nicotine. The inhibition was observed with DJ-7141 of more than 3 μM and the IC_{50} was ~10 μM. The inhibitory effect of this new compound was less than that of clonidine, the IC_{50} of which is ~3 μM. Neither DJ-7141 nor clonidine affected catecholamine secretion from the cells induced by high K^+.

We examined the effect of DJ-7141 on $[^{45}Ca]^{2+}$ uptake by the cells induced by nicotine, which is essential for initiation of catecholamine secretion. As shown in Fig. 3, DJ-7141 inhibited nicotine-induced $[^{45}Ca]^{2+}$ uptake and the concentration–response curve was similar to that for inhibition of catecholamine secretion.

Next, to clarify the mode of action of DJ-7141 on catecholamine secretion, we examined whether increase in the concentration of nicotine or Ca^{2+} in the medium could reverse the inhibitory effect of DJ-7141. As shown in Fig. 4, the effect of DJ-7141 in inhibiting nicotine-induced catecholamine secretion

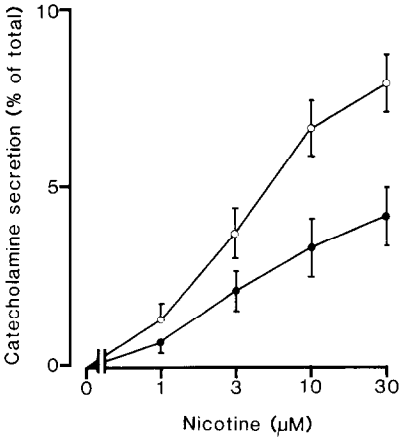

Fig. 4. Inhibitory effect of DJ-7141 on catecholamine secretion induced by various concentrations of nicotine. Cells were stimulated with various concentrations of nicotine in the absence (○) and presence (●) of DJ-7141 (10 μM). Catecholamine secretion is shown as a percentage of the total catecholamine in the cells. Points and bars are means and standard deviations of three or four experiments.

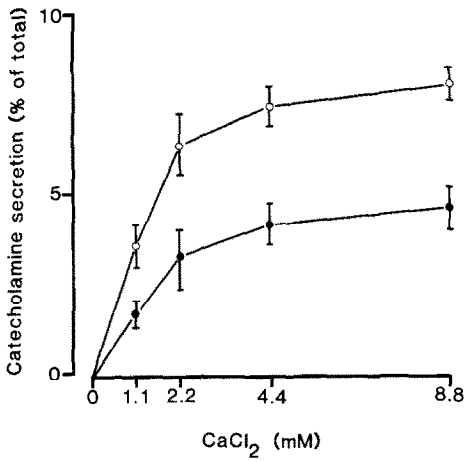

Fig. 5. Inhibitory effect of DJ-7141 on catecholamine secretion induced by nicotine with various concentrations of Ca^{2+}. Cells were stimulated with nicotine (10 μM) with various concentrations of Ca^{2+} in the absence (○) and presence (●) of DJ-7141 (10 μM). Catecholamine secretion is shown as a percentage of the total cellular catecholamine. Points and bars are means and standard deviations of three experiments.

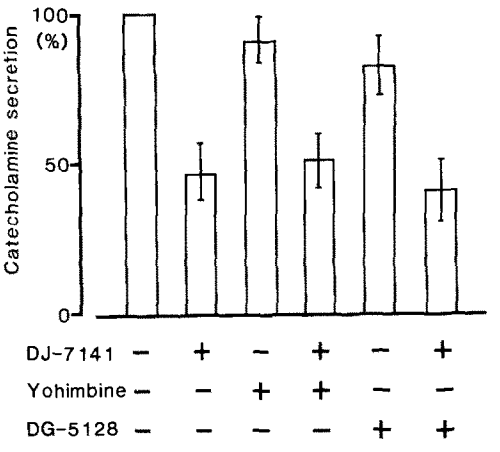

Fig. 6. Effects of yohimbine and DG-5128 on catecholamine secretion induced by nicotine and on the inhibitory effect of DJ-7141 on nicotine-induced catecholamine secretion. Cells were incubated with or without drugs (DJ-7141, 10 μM; yohimbine, 10 μM; DG-5128, 10 μM) and stimulated with nicotine (10 μM) as described in Materials and Methods. Catecholamine secretion induced by nicotine without drugs is expressed as 100%. Columns and bars are means and standard deviations of three or four experiments.

was not reversed by increasing the concentration of nicotine, suggesting that the effect of DJ-7141 was not due to competitive antagonism at the nicotinic ACh receptor site. Figure 5 shows the effect of DJ-7141 on nicotine-induced catecholamine secretion at different concentrations of extracellular Ca^{2+}. The inhibitory effect of DJ-7141 was not reversed by increasing the concentration of extracellular Ca^{2+}, suggesting that it was not due to antagonism of the Ca^{2+} channel.

Next, we examined whether the effect of DJ-7141 in inhibiting catecholamine secretion was mediated through its effect on the α_2-adrenoceptor, which may be present on adrenal medullary cells [6–11]. Figure 6 shows the effects of the α_2-antagonists yohimbine and DG-5128 [12] on nicotine-induced catecholamine secretion and on the inhibitory effect of DJ-7141 on nicotine-induced catecholamine secretion. Neither yohimbine nor DG-5128 significantly affected basal or nicotine-induced catecholamine secretion, and neither prevented the inhibitory effect of DJ-7141 on nicotine-induced catecholamine secretion. Thus an α_2-adrenoceptor regulating catecholamine secretion was probably not present on bovine adrenal medullary cells and the inhibition of catecholamine secretion by DJ-7141 was probably not mediated by an α_2-adrenoceptor.

DISCUSSION

The newly synthesized imidazole compound DJ-7141 (Fig. 1) is a unique α_2-adrenoceptor agonist that shows high affinity to peripheral α_2-adrenoceptors but has little affect on central α_2-adrenoceptors. Therefore, its hypotensive effect is less than that of another α_2-agonist, clonidine [1, 2]. In the present study we showed that this new α_2-agonist inhibited catecholamine secretion from isolated bovine adrenal medullary cells induced by nicotinic ACh receptor stimulation. This inhibitory effect of DJ-7141 was less than that of clonidine.

DJ-7141 inhibited nicotine-induced catecholamine secretion, but that not induced by high K^+, suggesting that DJ-7141 acts on a mechanism involving nicotinic ACh receptor on adrenal medullary cells. Moreover, DJ-7141 inhibited $[^{45}Ca]^{2+}$ uptake by the cells induced by nicotinic stimulation, which is of critical importance for initiation of catecholamine secretion. The concentration–response curve for inhibition of $[^{45}Ca]^{2+}$ uptake was similar to that for inhibition of catecholamine secretion. Therefore, the inhibition of catecholamine secretion by DJ-7141 seemed to be the result of inhibition of $[^{45}Ca]^{2+}$ uptake by the cells.

Next, we studied the modes of action of DJ-7141 in inhibiting catecholamine secretion and Ca^{2+} uptake by the cells. The inhibitory effect of DJ-7141 was not antagonized by increasing the concentration of nicotine or Ca^{2+} in the medium, suggesting that the inhibition by DJ-7141 is due to interference with coupling between stimulation of nicotinic ACh receptors and activation of Ca^{2+} channels.

Furthermore, we studied whether the inhibitory effect of DJ-7141 was mediated by the α_2-adrenoceptor. Recently, the presence of α_2-adrenoceptor on adrenal medullary cells and its role in regulation of catecholamine secretion has been discussed [13, 14]. If there is an α_2-adrenoceptor that causes inhibitory regulation of catecholamine secretion like that in adrenergic nerve terminals [15–17], an α_2-antagonist might stimulate the secretion of catecholamine and reverse the inhibitory effect of the α_2-agonist DJ-7141. However, the α_2-antagonists yohimbine and DG-5128 did not significantly affect basal or nicotine-induced catecholamine secretion.

Moreover, the inhibitory effect of DJ-7141 was not reversed by these α_2-antagonists. Recently, the inhibition by clonidine of catecholamine secretion from bovine adrenal medullary cells was also found not to be reversed by yohimbine and other α_2-antagonists [11, 14]. From these results, it is unlikely that there is an α_2-adrenoceptor that regulates catecholamine secretion on bovine adrenal medullary cells or that the inhibitory effect of DJ-7141 is mediated by an α_2-adrenoceptor. In bovine adrenal medullary cells, the inhibitory effect of DJ-7141 seems to be due to a non-specific effect on catecholamine secretion.

In conclusion, the present study demonstrated that the new α_2-adrenoceptor agonist DJ-7141 inhibits secretion of catecholamine from adrenal medulla. This effect may contribute, in part, to its hypotensive effect by reducing the circulating catecholamine level.

Acknowledgements—We express our appreciation to Mrs Keiko Tachibana for secretarial assistance. DJ-7141 and DG-5128 were kindly synthesized and donated by Daiichi Seiyaku Co., Tokyo, Japan.

REFERENCES

1. F. Ishikawa, M. Kitagawa, Y. Satoh, J. Saegusa, S. Tanaka, S. Shibamura and T. Chiba, *Chem. Pharmac. Bull., Tokyo* **33**, 2838 (1985).
2. I. Muramatsu, M. Oshita, S. Hashimoto and S. Kigoshi, *Japan. J. Pharmac.* **41**, 61 (1986).
3. M. Oka, M. Isosaki and N. Yanagihara, in *Catecholamines: Basic and Clinical Frontiers* (Eds E. Usdin, I. J. Kopin and J. Barchas), p. 70. Pergamon Press, Oxford (1979).
4. K. L. Kelner, R. A. Levine, K. Morita and H. B. Pollard, *Neurochem. Int.* 7, 373 (1985).
5. M. Oka, M. Isosaki and J. Watanabe, in *Advances in the Biosciences: Synthesis, Storage and Secretion of Adrenal Catecholamine* (Eds F. Izumi, M. Oka and K. Kumakura), Vol. 36, p. 29. Pergamon Press, Oxford (1982).
6. Y. Gutman and P. Boonyaviroj, *Eur. J. Pharmac.* **28**, 384 (1974).
7. K. Starke, B. D. Gorlitz, H. Montel and H. J. Schumann, *Experientia* **30**, 1170 (1974).
8. Y. Gutman and P. Boonyaviroj, *J. Neural Transm.* **40**, 245 (1977).
9. A. Greenberg and O. Zinder, *Cell Tissue Res.* **226**, 655 (1982).
10. A. Wada, S. Sakurai, H. Kobayashi, N. Yanagihara and F. Izumi, *Brain Res.* **252**, 189 (1982).
11. S. Sakurai, A. Wada, F. Izumi, H. Kobayashi and N. Yanagihara, *Naunyn-Schmiedeberg's Arch. Pharmac.* **324**, 15 (1983).
12. I. Muramatsu, M. Oshita and K. Yamanaka, *J. Pharmac. exp. Ther.* **227**, 194 (1983).
13. T. R. Sharma, T. D. Wakade, R. K. Malhotra and A. R. Wakade, *Eur. J. Pharmac.* **122**, 167 (1986).
14. D. A. Powis and P. F. Baker, *Molec. Pharmac.* **29**, 134 (1986).
15. K. Starke, *Rev. Physiol. Biochem. Pharmacol.* **77**, 1 (1977).
16. T. C. Westfall, *Physiol. Rev.* **57**, 659 (1977).
17. S. Z. Langer, *Pharmac. Rev.* **32**, 337 (1981).