PHARMACODYNAMIC ACTIONS OF (S)-2-[4,5-DIHYDRO-5-PROPYL-2 (3H)-FURYLIDENE]-1,3-CYCLOPENTANEDIONE (OUDENONE)

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Abstract—The pharmacodynamic actions of (S)-2-[4,5-dihydro-5-propyl-2(3H)-furylidene]-1,3-cyclopentanedione (oudenone) were studied in both anesthetized animals and isolated organs. Oudenone (10–40 mg/kg i.v.) induced an initial rise in blood pressure followed by a prolonged hypotension in the anesthetized rats. In unanesthetized spontaneously hypertensive rats (SHR), oudenone (5–200 mg/kg p.o.) caused a dose-related decrease in the systolic blood pressure. The initial pressor effect was diminished by pretreatments with phentolamine, guanethidine, hexamethonium and was abolished in the pithed rats. In addition, intracisternal administrations of oudenone (100–600 μ g/kg) showed a marked increase in blood pressure in the anesthetized rats, suggesting that the pressor effect may be due to centrally mediated actions. Oudenone, given intra-arterially into the femoral artery (400–800 μ g/kg), caused a long-lasting vasodilation in anesthetized dogs. At a relatively high dose (40 mg/kg i.v.), oudenone antagonized all pressor responses to autonomic agents and central vagus nerve stimulation in anesthetized rats and dogs, however, oudenone showed no anti-cholinergic, histaminergic, beta-adrenergic and adrenergic neuron blocking properties.

Tyrosine hydroxylase, which catalyzes the hydroxylation of tyrosine to form dihydroxyphenylalanine, is the rate limiting step in the catecholamine biosynthesis (1) and many substances have been tested as potential inhibitions of this enzyme (2). Recently, several new inhibitors of tyrosine hydroxylase and dopamine- β -hydroxylase from microbial origin have been discovered (3–10). Oudenone is one of these microbial inhibitors of tyrosine hydroxylase.

From biochemical experiments, oudenone has been reported to inhibit tyrosine hydroxylase both *in vitro* and *in vivo* and decreases endogenous catecholamine levels (3, 5), however, pharmacological actions of oudenone have yet to be clarified. The purpose of the present work was to investigate the pharmacological actions of oudenone (synthesized according to the procedure by Ohno et al. (4)) in detail and to elucidate the mechanisms whereby these actions were caused by oudenone.

MATERIALS AND METHODS

Experiments in the anesthetized normotensive rats

Male Wistar rats weighing 280–350 g were anesthetized with urethane (1.5 g/kg s.c.), the trachea cannulated and arterial blood pressure and heart rate were recorded from the right common carotid artery with a pressure transducer (Nihon Kohden, MPU-0.5) con-

nected to a polygraph (Nihon Kohden, RJB-3004). A small polyethylene tube was inserted into the left external jugular vein in order to inject drugs intravenously.

Experiments in the unanesthetized spontaneously hypertensive rats

In male spontaneously hypertensive rats weighing 270–300 g measurement of the systolic blood pressure was done using the tail-plethysmographic method (11). Rats were placed on a prewarm table (33–35°C) for 3 min and then moved to another table (37–39°C). The tails were positioned through an inflatable cuff which was stabilized in a plastic mold. Systolic blood pressure was measured with a pressure transducer (Shinkoh, LPU-0.1–350) and recorded on a polygraph (Nihon Kohden, RM-85).

Experiments in the adrenalectomized rats

Male Wistar rats weighing 300–330 g were anesthetized with urethane (1.5 g/kg s.c.), the trachea cannulated and acute bilateral adrenalectomy carried out through a dorsal mid-line incision. Arterial blood pressure and heart rate were recorded as described above.

Experiments in the pithed rats

Male Wistar rats weighing 300–350 g were anesthetized with ether, the trachea cannulated and the animal pithed through right orbit with a steel rod after which artificial respiration was commenced immediately. Arterial blood pressure and heart rate were recorded as described above.

Experiments in the intracisternal administrations

Male Wistar rats weighing 270–300 g were anesthetized with urethane (1.5 g/kg s.c.), the trachea cannulated and a fine polyethylene cannula was inserted into the cisternal magna through the punctured core at C₁–C₂ level in order to administer drugs intracisternally.

Experiments in the anesthetized cats

Cats of either sex weighing 2.8–3.5 kg were used. Anesthesia was induced with ether and maintained with α -chloralose (80 mg/kg i.v.) and urethane (500 mg/kg i.v.). The nictitating membrane stretched with a weight of 2 g was picked up with a force-displacement transducer (Nihon Kohden, S.B-1T) and recorded on a polygraph (Nihon Kohden, RJB-3004). The right superior cervical preganglionic sympathetic nerve was stimulated through platinum electrodes, with a supramaximal voltage (6–8V), at various frequencies which ranged from 0.3–30 Hz and squarewave pulses of 0.6 msec duration for 60 sec by an electronic stimulator (Nihon Kohden, MSE-3R). Drugs were administered into the brachial vein.

Experiments in the isolated rabbit jejunum

Rabbits of either sex weighing 2–3 kg were sacrificed by cervical dislocation and the jejunum was rapidly removed and placed in a dish containing Tyrode solution. The medium was maintained at $38 \pm 2^\circ\text{C}$ and was bubbled with 95% O₂ and 5% CO₂. According to the procedure by Finkleman (12), the nerve was stimulated through platinum electrodes, with a supramaximal voltage (15–20V) at a frequency 30 Hz and a duration of 0.5 msec for 4 seconds every 3 min by an electronic stimulator (Nihon Kohden, MSE-3R). The motility of the jejunum was recorded on a kymograph.

Experiments in the isolated rabbit aortic strips

Rabbits of either sex weighing 2–3 kg were sacrificed by cervical dislocation and the thoracic aorta was rapidly removed and placed in a dish containing Krebs-Henseleit solution. The medium was maintained at $37 \pm 2^\circ\text{C}$ and was bubbled with 95% O_2 and 5% CO_2 . The strip stretched with a weight of 1.5 g was picked up with a force-displacement transducer (Nihon Kohden, SB-1T) and recorded on a polygraph (Nihon Kohden, RJB-3004). Experiments were started after 1.5–2.0 hr during which time the bath medium was changed 3–4 times.

Experiments in the isolated perfused guinea pig heart preparations

Male guinea pigs weighing 300–350 g were stunned by a blow on the head. The hearts were removed and mounted in a manner similar to that described by Garb, S. et al. (13) and perfused at a constant pressure (60–80 mmHg) with Krebs-Henseleit solution bubbled with 95% O_2 and 5% CO_2 at $37 \pm 2^\circ$. The hearts were perfused for 30 min before experiments were begun and during this time, rate, flow and contraction had become stable. The contraction and heart rate were recorded by a force-displacement transducer (Nihon Kohden, MPU-0.5). Oudenone was administered directly into the perfusion cannula.

Experiments in the perfused hind-leg of the dog

Dogs of either sex weighing 7–15 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and supplemental doses of 5 mg/kg i.v. per hour. Sodium heparin, 300 U/kg i.v. was given initially and 100 U/kg i.v. was added at 1 hr intervals. The right femoral artery was then perfused with blood led from the right common carotid artery. A perfusion pump (Central Kagaku Boheki, Ismeter MP-4) was set just proximal to the pressure transducer (Nihon Kohden, MPU-0.5), these being linked through the circuit of the polyethylene tube. Femoral blood flow of the pump was adjusted to the flow before treatment for performing the constant flow perfusion. Systemic blood pressure was obtained from the left femoral artery and at the same time, the perfusion flow rate was monitored on a polygraph (Nihon Kohden, RJB-3004). The 0.1 ml of the solution containing oudenone was administered into the femoral artery through the polyethylene tube previously attached to a perfusion pump.

Experiments in the anesthetized dogs

Dogs of either sex weighing 6–17 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and supplemental doses of 5 mg/kg i.v. per hour. The carotid pressor reflex was elicited by bilateral clamping of the common carotid arteries for 20 sec. Stimulation of the central stump of the vagus nerve was carried out by using platinum electrodes with a voltage (50 v), at a frequency of 50 Hz and the duration of 1 msec for 10 sec by an electronic stimulator (Nihon Kohden, MSE-3R). Stimulation of the peripheral stump of the vagus nerve was carried out with a voltage (30v), at a frequency of 50 Hz and a duration of 0.5 msec for 3 sec. Systemic blood pressure was recorded as described above and drugs were injected into the axial vein. All drugs were dissolved in 0.9% NaCl and were expressed in terms of salts (except oudenone). Statistical significance of difference was evaluated by Student's *t*-test.

Drugs used; (dl)-norepinephrine hydrochloride (Sankyo), (dl)-epinephrine hydrochloride (Sankyo), guanethidine monosulfate (CIBA), phentolamine mesylate (CIBA), hexamethonium bromide (Yamanouchi), reserpine (Daiichi), cocaine hydrochloride (Dainippon), acetylcholine chloride (Daiichi), histamine dihydrochloride (Wako), dimethylphenylpiperazinium iodide (Aldrich), (1)-isoproterenol hydrochloride (Nikken), tyramine hydrochloride (Daiichikagaku), angiotensin (CIBA), oudenone (synthesized by Dr. T. Masuda of Takeda Chemical Industries, LTD.)

RESULTS

Effects of oudenone on blood pressure and heart rate in the anesthetized rats (Fig. 1)

Oudenone (10–40 mg/kg i.v.) produced an initial rise in blood pressure followed by a prolonged fall in a dose-dependent manner. The administration of oudenone (10 mg/kg i.v.) did not cause a significant change in blood pressure and heart rate. With higher doses (20 and 40 mg/kg i.v.), the pressor phase was more prominent and the depressor phase was more obvious and long-lasting. The initial pressor effect reached its maximal levels at 11 ± 2 mmHg (20 mg/kg) and 21 ± 3 mmHg (40 mg/kg), respectively about 5 min after

FIG. 1. Effects of intravenous administrations of oudenone on blood pressure and heart rate in anesthetized rats. Values represent means $\pm$ standard errors. Numbers in parentheses indicate the number of animals. $\square$ — $\square$; 10 mg/kg i.v. $\circ$ — $\circ$; 20 mg/kg i.v., $\bullet$ — $\bullet$; 40 mg/kg i.v.

FIG. 2. Percent changes in the systolic blood pressure in the unanesthetized SHR after oral administrations of oudenone. Values represent means $\pm$ standard errors. Five rats were used for each dose. $\bullet$ — $\bullet$; 5 mg/kg p.o., $\circ$ — $\circ$; 10 mg/kg p.o., $\square$ — $\square$; 100 mg/kg p.o., $\bullet$ — $\bullet$; 200 mg/kg p.o.

administrations of oudenone. On the other hand, the levels of hypotension were 11 ± 3 mmHg (20 mg/kg) and 19 ± 2 mmHg (40 mg/kg), respectively 4 hr after administrations. In both doses, gradual increases in heart rate were also observed.

Effects of oudenone on the systolic blood pressure in the unanesthetized SHR (Fig. 2)

Oudenone (5–200 mg/kg p.o.) reduced the systolic blood pressure dose-dependently. The hypotensive effect became apparent within 1 hr and reached its maximum 3 hr after administration. At the lowest dose (5 mg/kg), the hypotensive effect recovered within 7 hr after administration, however, as the dose was increasing, the duration of the depressor phase was more prolonged and distinctive. The average systolic blood pressure of 5 rats of 192 ± 5 mmHg, prior to administration, was lowered to 144 ± 3 mmHg (about 25% depression compared with the control) after 100 mg/kg p.o. of oudenone, while 200 mg/kg p.o. of oudenone decreased the systolic blood pressure from 193 ± 5 mmHg to 134 ± 3 mmHg (about 31% depression). In these cases, recovery of hypotension was not observed within the experimental periods.

Effects of reserpine and cocaine on the pressor response to oudenone (Fig. 3)

As shown in Fig. 3, the pressor response to oudenone (40 mg/kg i.v.) was considerably diminished after treatment with reserpine (1 mg/kg/day s.c. 2 days and 5 hours prior to experiments) and the onset of the hypotension was accelerated. On the other hand, the

FIG. 3. Effects of cocaine and reserpine pretreatments on the pressor response to oudenone in the anesthetized rats. Values represent means $\pm$ standard errors. Numbers in parentheses indicate the number of animals. ●—●; reserpine pretreatment, ○—○; cocaine pretreatment, ○—○; control (oudenone 40 mg/kg i.v.)

FIG. 4. Effects of phentolamine (Phe), guanethidine (Gua) and hexamethonium (Hex) pretreatments on the pressor response to oudenone. Values represent means $\pm$ standard errors. Student's *t*-test, *p* values in the figure. Number in parentheses indicate the number of animals. control; oudenone 40 mg/kg i.v.

response was increased after treatment with cocaine (1 mg/kg i.v. 1 hr prior to 40 mg/kg i.v. administration of oudenone). In this case, the onset of the hypotension was decelerated.

Effects of drugs on the pressor response to oudenone (Fig. 4)

As shown in Fig. 4, the pressor response to oudenone (40 mg/kg i.v.) was depressed by pretreatments with phentolamine (1 mg/kg i.v.), guanethidine (10 mg/kg i.v.) and hexamethonium (10 mg/kg i.v.). In these groups of experiments, the blocking action of phentolamine was most pronounced though the differences were not significant ($p > 0.05$).

Effects of oudenone on blood pressure in the adrenalectomized rats and in the pithed rats (Table 1)

Bilateral adrenalectomy did not suppress the pressor response to oudenone (40 mg/kg i.v.), however, a statistically significant disappearance of the response was observed in the pithed rats ($p < 0.01$).

TABLE 1. Blood pressure response to oudenone in intact (control), adrenalectomized and pithed rats

Treatment	Increase in blood pressure (mmHg)
Control	$20.71 \pm 3.32(7)$
Adrenalectomy	$22.40 \pm 4.23(5)$
Pithed	* $3.50 \pm 1.09(6)$

Values represent means $\pm$ standard errors. Oudenone (40 mg/kg) was given i.v. in all preparations.

* Significantly different from the control ($p < 0.01$)

Intracisternal administrations of oudenone in the anesthetized rats (Fig. 5)

Intravenous administration of 600 μ g/kg of oudenone caused no change in blood pressure and heart rate. Intracisternal (i.c.) administration of 0.01 ml/kg of 0.9% NaCl led to only a small and transient fall in blood pressure. Administrations of oudenone (100–600 μ g/kg) intracisternally produced a distinct rise in blood pressure and the pressor response reached its maximal level 60–120 sec after administration of oudenone, which lasted for 30–120 min, dose dependently. An increase in heart rate also occurred and lasted for the experimental periods.

Effects of oudenone on the contraction of nictitating membrane elicited by electrical stimulation (Fig. 6)

As shown in Fig. 6, administration of oudenone (40 mg/kg i.v.) to 5 anesthetized cats produced no significant influence upon the contraction of nictitating membrane elicited by electrical stimulation at various frequencies.

Effects of oudenone on the decreasing motility of the rabbit jejunum elicited by electrical stimulation (Fig. 7)

The inhibitory effect of stimulating the visceral nerve on the pendular movement of isolated rabbit jejunum was not abolished after adding oudenone in concentrations of

FIG. 5. Effects of intracisternal administrations of oudenone on blood pressure and heart rate in anesthetized rats. BP; blood pressure, HR; heart rate

FIG. 6. Effects of oudenone on responses of cat nictitating membrane contraction elicited by electrical stimulation at various frequencies. Ordinate refers to percent of control 3 hours after administrations of oudenone (40 mg/kg i.v.). Values represent means $\pm$ standard errors. $\bullet$ — $\bullet$: control (n=5) $\circ$ — $\circ$: after administrations of oudenone (n=5)

FIG. 7. Effects of oudenone on adrenergic nerve stimulation which decreased motility of isolated rabbit jejunum. Oudenone was added to the fluid at arrows.

3×10^{-6} to 3×10^{-4} g/ml to the bath fluid, however, oudenone decreased the pendular movements, dose dependently.

Effects of oudenone on the heart rate and contraction of the isolated perfused guinea pig hearts and on the tension of the rabbit aortic strips (Fig. 8)

Although the addition of 10^{-5} g of oudenone to the guinea pig hearts exerted no effects on either heart rate or force of contraction, negative chronotropic and inotropic effects were concentration-dependent between 3×10^{-5} and 10^{-3} g (Fig. 8A). The addition of oudenone (3×10^{-7} – 10^{-3} g/ml) to the medium in which the rabbit aortic strips was suspended, caused a relaxation in a dose-dependent manner (Fig. 8B)

FIG. 8. (A) Effects of ouidenone on the contractile force and heart rate in the isolated perfused guinea pig hearts. CF; contractile force, HR: heart rate (B) Effects of ouidenone on the tension of rabbit aortic strips.

FIG. 9. Effects of ouidenone on the femoral perfusion pressure in anesthetized dogs. Values represent means±standard errors. Numbers in parentheses indicate number of animals. ○—○; 400 µg/kg i.a., ●—●; 800 µg/kg i.a.

FIG. 10. Effects of ouidenone on responses to adrenergic, sympathetic and parasympathetic activations in anesthetized dogs. Numbers across the top refer to magnitude of responses (mean blood pressure, mmHg) prior to administration of ouidenone. Bars refer to change in these response 3 hr after ouidenone and numbers near the base of bars indicate the number of dogs. Values represent means ±standard errors. NE; norepinephrine (3 µg/kg i.v.), PVS; peripheral vagus stump stimulation, CVS; central vagus stump stimulation, COR; carotid occlusion. (See methods for details.) * Significantly different from the controls (p<0.01)

Effects of oudenone on the femoral perfusion pressure in the anesthetized dogs (Fig. 9)

Intra-arterial administrations of oudenone (400–800 $\mu\text{g/kg}$) into the femoral artery caused a transient fall immediately after administration (not shown in the figure) followed by a prolonged reduction in the femoral perfusion pressure. Under these experimental conditions, no significant alternation in the systemic blood pressure could be observed.

Effects of oudenone on responses to norepinephrine, bilateral common carotid artery occlusion and vagal stimulation in the anesthetized dogs (Fig. 10)

Effects of intravenous administrations of oudenone (20–40 mg/kg) on vasomotor responses to norepinephrine, carotid occlusion, centrifugal and centripetal vagal stimulations in the anesthetized dogs are summarized in Fig. 10. Although the depressor response induced by centrifugal vagal stimulation was not altered by oudenone (20–40 mg/kg), statistically significant reduction to the pressor responses was caused by oudenone (40 mg/kg) ($p < 0.01$)

Effects of oudenone on the autonomic responses in the anesthetized rats (Fig. 11)

Several vasomotor responses to autonomic agents were tested. These include intravenous administrations of acetylcholine (10 $\mu\text{g/kg}$), histamine (5 $\mu\text{g/kg}$), norepinephrine (5 $\mu\text{g/kg}$), epinephrine (3 $\mu\text{g/kg}$), tyramine (1 mg/kg), isoproterenol (0.3 $\mu\text{g/kg}$), angiotensin (1 $\mu\text{g/kg}$) and dimethylphenylpiperazinium (200 $\mu\text{g/kg}$) (Fig. 11.). Oudenone (20–40 mg/kg i.v.) caused no alternations of the responses to acetylcholine, histamine and isoproterenol, however, all pressor responses were significantly depressed by oudenone (40 mg/kg i.v.) ($p < 0.01$).

FIG. 11. Effects of oudenone on responses to autonomic agents in anesthetized rats.

Numbers across the top refer to magnitude of responses (mean blood pressure, mmHg) prior to administration of oudenone. Bars refer to change in these responses 3 hr after oudenone and numbers near the base of the bars indicate the numbers of rats. ACh; acetylcholine (10 $\mu\text{g/kg}$ i.v.), Hist; histamine (5 $\mu\text{g/kg}$ i.v.), NE; norepinephrine (5 $\mu\text{g/kg}$ i.v.), E; epinephrine (3 $\mu\text{g/kg}$ i.v.), Tyr; tyramine (1 mg/kg i.v.), Iso; isoproterenol (0.3 $\mu\text{g/kg}$ i.v.), DMPP; dimethylphenylpiperazinium (200 $\mu\text{g/kg}$ i.v.), Ang; angiotensin (1 $\mu\text{g/kg}$ i.v.)

* Significantly different from the controls ($p < 0.01$)

DISCUSSION

The results presented in this paper show that intravenous administrations of oudenone induced an initial rise in blood pressure followed by a prolonged hypotension in the anesthetized normotensive rats and that oral administrations of oudenone caused a dose-related decrease in the systolic blood pressure in the unanesthetized SHR. Experiments were done to determine whether or not the pressor action is mediated through adrenergic mechanisms in the peripheral sites. That oudenone induced negative inotropic and chronotropic effects in the isolated guinea-pig hearts, suggests that release of catecholamines from the store sites may not be the cause of the pressor effect. Furthermore, release of catecholamines from the adrenal medulla can hardly be attributed to the pressor response, because bilateral adrenalectomy of the rats had no influence on this response. The depression of the response by phentolamine, guanethidine and hexamethonium and that in the pithed rats, suggests that the response is due rather to central actions. The most significant evidence for this assumption is that an increase in blood pressure was induced by intracisternal administrations of small doses of oudenone with which intravenous administrations of the same doses had no effects systemically.

It is well-known that cocaine inhibits the uptake mechanism which inactivates both endogenous and exogenous catecholamines and results in higher accumulation of active amines at the receptor sites. The enhancement of the pressor response to oudenone by cocaine suggests that catecholamines are released from the peripheral nerve ending by centrally mediated actions of oudenone. This is further supported by the results that the pressor response was considerably diminished after reserpine treatment. This is understandable if we consider that reserpine leads to a depletion of catecholamines both in the central sympathetic nerve fibers and in the peripheral nerve fibers. Although the discharge frequency in the preganglionic sympathetic fibers may be increased, the lack of transmitters in the post-ganglionic fibers with reserpine seems to be decisive for the decrease of the pressor response to oudenone.

From the results described above, it is firmly concluded that the pressor response to oudenone is explained by the centrally mediated actions, however, it is still obscure by which pathway the stimulation of the central elements leads to an increase in blood pressure. The following possibilities are considered: 1) stimulation of the central muscarinic mechanism; Brezenoff (14) reported that eserine, an inhibitor of acetylcholine esterase produced a pressor response *via* central muscarinic mechanisms and the active participation of brain acetylcholine has also been reported (15). 2) depletion of brain serotonin; p-chlorophenyl-alanine, a potent inhibitor of tryptophan hydroxylase (16-19), has been reported to elevate blood pressure by selective depletion of serotonin in the brain (20). 3) stimulation of central β -adrenoceptors; Day and Roach (21-22) reported that centrally administered isoproterenol increased blood pressure *via* central β -adrenoceptors.

When considering of the effect of oudenone in lowering blood pressure, certain modes of action seem relatively insignificant. Under the experimental conditions, oudenone caused no significant changes in the responses to histamine, acetylcholine, centrifugal stimu-

lation of the vagus nerve and isoproterenol, therefore it seems that oudenone apparently does not possess anti-histaminergic, anti-cholinergic and beta-adrenergic blocking properties. Likewise, direct evidence for the lack of adrenergic neuron blocking properties was obtained by the results that oudenone had no effects on the contraction of the cat nictitating membrane elicited by the stimulation of the preganglionic cervical sympathetic nerve and that of the rabbit jejunum elicited by the stimulation of the postganglionic sympathetic nerve. There are certain actions that must be taken into account when evaluating the hypotensive effect of oudenone. The results that oudenone caused relaxations in the rabbit aortic strips and reduced the femoral perfusion pressure, suggest that oudenone possesses a vasodilatory action. In this case, the latter effect of oudenone in reducing the femoral perfusion pressure was long-acting. Furthermore, oudenone antagonized effectively all the pressor responses to autonomic agents and centripetal stimulation of the vagus nerve. These two effects could be taken as an indicator of the hypotensive effect of oudenone.

From the results obtained in this paper only, it is difficult to show any direct evidence that the hypotension with oudenone is due to decrease of the endogenous catecholamine levels by inhibiting tyrosine hydroxylase. Since oudenone showed no adrenergic neuron blocking properties even at a relatively high dose, it seems unlikely that decrease in the catecholamine content would play a role in the production of hypotension of oudenone.

In conclusion, the findings herein indicate that the initial pressor response of oudenone may have its origin in the central nervous system and that the hypotensive effect of oudenone is due to a long lasting vasodilation and a non-specific antagonistic effect on the responses to all the pressor substances.

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