ACTION OF DEPRESSOR-I, A HYPOTENSIVE PHOSPHOLIPID FROM BOVINE BRAIN, ON SYSTEMIC AND ARTERIAL BLOOD PRESSURES OF VARIOUS SPECIES

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Abstract—Effects of “Depressor-I” (D-I), a new hypotensive phospholipid obtained from bovine brain lipid fraction, on systemic arterial blood pressure were investigated. The hypotensive activity of D-I in urethane anaesthetized rats was dose dependent and tachyphylaxis and/or sensitization were not observed. Increments of the respiration and the heart rate were observed with sharp falls in blood pressures following intravenous administration of D-I, in simultaneous recordings in anaesthetized rats. D-I elicited hypotension in all species of animals examined, and the sensitivities to D-I were much the same, however, there were two types in patterns of duration on responses and the durations were also dose dependent. D-I exhibited depressor-responses even in conscious rats, though responses were much smaller compared with those seen in anaesthetized rats. In a comparison of anaesthetic agents in rats, the highest hypotensive activity of D-I was observed with pentobarbital anaesthesia, a moderate response was seen with α-chloralose and the least response was seen with urethane. In spinal rats or those pretreated with reserpine or antagonists, such as atropine, diphenhydramine, propranolol and hexamethonium, D-I also elicited hypotension. These results suggest that “Depressor-I” does not elicit the depressor action via the stimulation of the central and the autonomic nervous systems but rather by a direct action on peripheral blood vessels.

With the development of lipid chemistry, pharmacological studies on biological active substances, particularly prostaglandins (PGs) have been documented by different groups. Recently, we reported the occurrence of a new hypotensive factor in bovine brain lipid fraction which was distinguishable from PGs and from known hypotensive agents (1–3). The chemical structure is now being investigated extensively. This active principle was tentatively termed “Depressor-I” (D-I) by the authors. The preparation of D-I was highly purified and showed a single spot on thin layer chromatography (TLC). When 10 μgs of the purified preparation per kg animal body weight was given i.v., there was a sharp fall of the arterial blood pressure of anaesthetized cat or rat. It is likely that D-I belongs to the phospholipid group and does resemble lysolecithin in certain chemical characteristics, however, a positive reaction against 2,4-dinitrophenylhydrazine reagent was seen on TLC and there was a significantly higher hypotensive activity than lysolecithin. In the present work, we examined the effects of D-I on the systemic arterial blood pressures of various species and conditions.
MATERIALS AND METHODS

Chemicals: The drugs used were obtained from the following sources: Urethane, histamine dihydrochloride and diphenhydramine hydrochloride (Wako), α-chloralose, DL-isopreterenol hydrochloride and tyramine hydrochloride (Nakarai), acetylcholine chloride and reserpine (Daiichi), sodium pentobarbital (Dainippon), hexamethonium bromide (Yamanouchi), DL-propranolol hydrochloride (Sumitomo), atropine sulfate (Tokyo Kasei), 1,1-dimethyl-4-phenylpiperazinium iodide (Aldrich).

Preparation of D-I: The preparation of D-I from bovine brain has already been reported (1), and a further modification was made to acquire a highly purified material showing a single spot on TLC, only using column chromatographic techniques (2). The modified procedure is as follows: Fresh bovine brain cut into pieces was homogenized with 10 liters of CHCl₃-MeOH (2:1, v/v) per kg wet tissue in a blender and then centrifuged. The residue was re-extracted in the same manner. The CHCl₃-MeOH extracts combined were washed by mixing with one-fifth the volume of 0.7% NaCl solution to eliminate the non-lipidal substances (4), and the lower phase was evaporated in vacuo. The CHCl₃-MeOH extract I (total lipid fraction, approx. 230 g/kg wet tissue) was extracted twice with 5 liters of acetone, and the residue was further washed twice with 500 ml of acetone. The acetone extracts combined and evaporated (acetone extract II, about 50 g/kg wet tissue) were further partitioned between equal volumes of 70% ethanol and hexane to eliminate inactive simple lipids. The aqueous ethanolic layer was evaporated in vacuo (crude extract III, approx. 1.2 g/kg wet tissue). The crude extract III (5.28 g corresponding to 4.40 kg of wet tissue) was applied to the first silicic acid column (Mallinckrodt, 100 mesh, activity II B, 100 g and hyflosuper-cel 50 g, 4.1 x 45 cm) and eluted with hexane, hexane-CHCl₃ (1:1, v/v), CHCl₃, CHCl₃-MeOH (8:2, v/v), CHCl₃-MeOH (6:4, v/v), CHCl₃-MeOH (4:6, v/v), MeOH-I, MeOH-II and MeOH-H₂O (1:1 v/v) successively by discontinuous gradient elution. Fractions of 1 liter were collected. Aliquots of the respective fractions were dried for determination of hypotensive activity and phosphorus contents. The hypotensive activity was detected in the eluate of CHCl₃-MeOH (4:6, v/v) (active fraction IV). The active fraction IV equivalent to 4.20 kg of wet tissue (299.7 mg) was subjected to the second silicic acid chromatography (Mallinckrodt, 100 mesh, II B 40 g, and hyflosuper-cel 10 g, 2.5 x 26 cm) and eluted with CHCl₃-MeOH (6:4, v/v) (400 ml), CHCl₃-MeOH (5:5, v/v) (600 ml), CHCl₃-MeOH (4:6, v/v) (400 ml) and MeOH (400 ml) by discontinuous gradient elution. The hypotensive activity was distributed in the eluate from 700 to 900 ml (active fraction V).

Subsequently the active fraction V (41.8 mg) was applied on a Sephadex LH-20 column (Pharmacia, 100 g, 2.7 x 90 cm) and eluted with CHCl₃-MeOH (1:1, v/v) and the active fraction VI was obtained (35.7 mg). Finally the fraction (32.0 mg) was further purified by a second gel filtration on a different Sephadex LH-20 column (100 g) and was eluted with acetone-ethanol (1:1, v/v), thus producing the active fraction VII (18.0 mg). The purification procedure is summarized in Table 1.

D-I seems to be a minor component in bovine brain lipid fraction as it corresponds to only 0.002% of the total lipid (CHCl₃-MeOH extract I). On TLC it shows characteristic
properties of choline-containing phospholipid, and closely resembles lysolecithin. However, there was a positive reaction against 2,4-dinitrophenylhydrazine or 2',7'-dichlorofluorescein reagent. In the analysis (3) the molar ratios of phosphorus: glycerol: choline-fatty acid were approx. 1:1:1:1. The most predominant fatty acid was oleic acid (56.1%) followed by palmitic acid (26.6%), eicosaenoic acid (8.5%) and stearic acid (6.4%). In the purification procedures, D-I became relatively labile. D-I is relatively soluble in water as well as in ethanol, methanol and chloroform.

**TABLE 1. Purification procedure of the hypotensive factor D-I**

| Stage | Weight (mg/kg wet tissue) | Phosphorus (μg/kg wet tissue) | Hypotensive activity (U/kg wet tissue)* |
|-------|--------------------------|-------------------------------|----------------------------------------|
| Crude extract (aq. EtOH extract) III | $1.2 \times 10^3$ | $11.34 \times 10^1$ | — |
| First silicic acid chromatography IV | 71.3 | 835 | 630 |
| Second silicic acid chromatography V | 11.0 | 576 | 610 |
| First gel filtration on Sephadex LH-20 (CHCl₃-MeOH, 1:1 v/v) VI | 9.4 | 482 | 600 |
| Second gel filtration on Sephadex LH-20 (acetone-EtOH, 1:1 v/v) VII | 5.3 | 271 | 540 |

Data are the mean of two experiments. Bioassays were carried out on urethane anaesthetized rats. *One unit (U) of D-I activity was defined as the amount required to produce a depressor response equal in amplitude to that produced by i.v. administration of $5 \times 10^{-7}$ g/kg of acetylcholine into an urethane anaesthetized rat weighing 250 g.

Blood pressure measurements in anaesthetized animals: Male Wistar rats weighing 250 g were anaesthetized with urethane (1.8 g/kg body weight, i.p.), $\alpha$-chloralose (80 mg/kg, i.p.) or sodium pentobarbital (40 mg/kg, i.p.). Guinea pigs (Hartley strain), cats, rabbits and dogs were anaesthetized with sodium pentobarbital (40 mg/kg, i.p.). The left carotid artery or a left femoral artery was catheterized, and connected to a direct writing mercury manometer or a transducer (Nihon Kohden MPU-0.5) connected to a multipurpose polygraph (Nihon Kohden RM-45). After dissolution with 0.15 ml saline (0.9 w/v % NaCl solution), samples were injected into a right femoral vein through a cannula which was flushed through immediately with 0.1 ml of saline.

Conscious rats: Conscious rats were prepared according to the method described by Weeks and Jones (5) and by Mizogami et al. (6). Under ether anaesthesia, Weeks catheters were inserted into the left abdominal aorta for measurement of the arterial blood pressure, and into a right abdominal vein for injections of samples. The cannulae, which were pre-filled with heparin solution (200 units/ml saline), were passed beneath the skin, brought out at the back of the neck and plugged with stainless stylettes. The arterial blood pressure was measured at least four days after preparation.

The abdominal arterial blood pressure was recorded using a pressure transducer connected to a multipurpose polygraph.

Spinal rats: The animals were anaesthetized with urethane (1.8 g/kg, i.p.), artificially ventilated, the cord cut at C2 position and the brain destroyed (7) using a modification of
the method described by Kumagai et al. (8). Blood pressure was recorded from a left carotid artery.

Reserpinized rats: Rats were reserpinized by giving 1 mg/kg/day of reserpine i.p. for four days and the animals were anaesthetized with urethane (1.8 g/kg, i.p.). The respiration was recorded using pick-up of thoracic breathing (Nihon Kohden MCR-2TA) on a multipurpose polygraph. The heart rate was also recorded on a multipurpose polygraph.

RESULTS

The dose-response relationship of the purified D-I preparation was examined on urethane anaesthetized rats in the range of $1.5 \times 10^{-5}$ to $3 \times 10^{-3}$ g/kg doses as shown in Fig. 1(B). Acetylcholine was also used for comparison [Fig. 1(A)]. The threshold dose of the purified D-I preparation in urethane anaesthetized rats was approx. $1.5 \times 10^{-5}$ g/kg on a weight basis and it corresponded to $7.8 \times 10^{-7}$ g/kg of phosphorus. In the range of $1.5 \times 10^{-5}$ to $3 \times 10^{-3}$ g/kg the depressor responses were parallel to the dosages. The maximal hypotensive effect of D-I was about 60 mmHg and approx. equal to the maximal effect obtained by acetylcholine. Such maximal value by the latter was shifted toward the right indicating that larger amounts of the D-I preparation were required to obtain the maximal hypotensive

![Fig. 1. Dose-response curves for acetylcholine and purified D-I preparation. The rats were anaesthetized with urethane (1.8 g/kg, i.p.). Each point represents the means ± s.e. (n=3). Ordinate: decrease in carotid arterial blood pressure in mmHg. Abscissa: (A) dose of acetylcholine in g/kg injected i.v. (B) dose of purified D-I preparation (active fraction VII) in g/kg given i.v.](image-url)
activity of the latter. It is noteworthy that the steeper slop was observed in D-I preparation.

As summarized in Table 2, 16±1.3 mmHg of decrease of blood pressure was recorded by giving i.v. 5×10⁻⁸ g/kg of acetylcholine. On the other hand, a dose of D-I which exhibited a depressor response to the same extent as that seen with acetylcholine in an urethane anaesthetized rat weighing 250 g was expressed as one unit (U) (4 U/kg). One unit corresponded to 9.8×10⁻⁶ g of the purified D-I preparation and contained approx. 5.0×10⁻⁷ g of phosphorus. The carotid arterial blood pressure, the respiration and the heart rate were measured simultaneously in pentobarbital anaesthetized rats, as indicated in Fig. 2. The heart rate and the respiration were increased by injections of 4 U/kg (39.2×10⁻⁶ g/kg) and 12 U/kg (117.6×10⁻⁶ g/kg) of D-I respectively. Neither tachyphylaxis nor sensitization was observed at 5 min intervals, as shown in Fig. 3. Effects of D-I on the systemic arterial blood pressure of rats, cats, dogs, rabbits and guinea pigs under pentobarbital anaesthesia are shown in Table 3 and Fig. 4. D-I produced sharp falls of blood pressure in all animals tested. In

![Fig. 2. Simultaneous recordings of the effect of D-I on the systemic arterial blood pressure, the respiration and the heart rate. The rat was anaesthetized with sodium pentobarbital (40 mg/kg, i.p.) and given i.v. 4 U/kg (39.2×10⁻⁶ g/kg) and 12 U/kg (117.6×10⁻⁶ g/kg) of purified D-I preparation at the points indicated by arrows. The blood pressure, respiration and heart were recorded on a multi-purpose polygraph.](image-url)
FIG. 3. Blood pressure responses to D-I in an unconscious rat. The rat weighing 250 g was anaesthetized with sodium pentobarbital (40 mg/kg, i.p.). Four U/kg (39.2 × 10^{-6} g/kg) of purified D-I preparation was injected i.v. at the points indicated by arrows. Blood pressure responses were recorded on a multipurpose polygraph.

| Species  | Effect    | mmHg |
|----------|-----------|------|
| Rat      | Hypotensive | 25 ± 3 |
| Cat      | Hypotensive | 23 ± 3 |
| Dog      | Hypotensive | 18 ± 4 |
| Rabbit   | Hypotensive | 27 ± 3 |
| Guinea pig | Hypotensive | 27 ± 3 |

Sodium pentobarbital (40 mg/kg, i.p.) was given. Purified D-I preparation 4 U/kg (39.2 × 10^{-6} g/kg) was also given i.v. The results are the means ± s.e. (n=3).

FIG. 4. Blood pressure responses to D-I in various species. Respective animal species were anaesthetized with sodium pentobarbital (40 mg/kg, i.p.), and 4 U/kg 39.2 × 10^{-6} g/kg and 12 U/kg (117.6 × 10^{-6} g/kg) of purified D-I preparation were given i.v. at the points indicated by arrows. Blood pressure responses were recorded on a smoked drum connected to a mercury manometer. A: male rat, B: female cat, C: male dog, D: male rabbit, E: male guinea pig.
comparison of responses to D-I in the animals, fall in blood pressure was limited to 18 to 27 mmHg with doses of 4 U/kg (39.2 \times 10^{-6} g/kg) of D-I. In pentobarbital anaesthetized rats, the blood pressure was sharply reduced approx. 25 mmHg and lasted about 1–2 min.

The cats, dogs and rats all behaved in a similar manner. In the rabbits and guinea pigs the fall in pressure lasted about 3–4 min then slowly returned to the original levels. The intravenous administrations of D-I in conscious, freely moving rats produced the same sharp falls in blood pressure as observed in the anaesthetized rats, although the responses were generally much smaller than those in anaesthetized animals (Fig. 5). A comparison was made between depressor responses to D-I in conscious rats and those in rats anaesthetized with urethane, \(\alpha\)-chloralose and sodium pentobarbital. The results are shown in Fig. 6.

**Fig. 5.** Blood pressure responses to D-I in a conscious rat. Eight U/kg (78.4 \times 10^{-6} g/kg) and 40 U/kg (392.0 \times 10^{-6} g/kg) of purified D-I preparation were injected i.v. into a freely-moving rat at the points indicated by arrows and recorded on a multipurpose polygraph.

**Fig. 6.** Variation of hypotensive activity of D-I in conscious and anaesthetized rats. Results represent the means \pm s.e. (n=5). Ordinate: decrease in abdominal arterial blood pressure in mmHg. A: conscious rats. B: rats anaesthetized with sodium pentobarbital 40 mg/kg given i.p. C: rats anaesthetized with \(\alpha\)-chloralose 80 mg/kg given i.p. D: rats anaesthetized with urethane 1.8 g/kg given i.p. Eight U/kg (78.4 \times 10^{-6} g/kg) of purified D-I preparation and \(5 \times 10^{-7} \) g/kg of acetylcholine given i.v.
FIG. 7. Effect of reserpine on the hypotensive activity induced by D-1. Rats were anaesthetized with urethane (1.8 g/kg, i.p.) and $3 \times 10^{-4}$ g/kg of tyramine hydrochloride (Ty) and 8 U/kg ($78.4 \times 10^{-6}$ g/kg, D-1) of purified D-1 preparation were given i.v. at the points indicated by arrows. The blood pressure responses were recorded on a smoked drum connected to a mercury manometer. Upper record: blood pressure responses in a normal rat. Lower record: blood pressure responses in a reserpinized rat.

FIG. 8. Effects of four antagonists on hypotensive activity of D-1 in urethane anaesthetized rats. The effect of each antagonist was confirmed by i.v. administration of the corresponding agonist, before and after injections of 8 U/kg ($78.4 \times 10^{-6}$ g/kg, i.v.) of purified D-1 preparation. Results represent the means±s.e. (n=3). Ordinate: change in carotid arterial blood pressure in mmHg. White column represents blood pressure response before administration of respective antagonist. Black column represents blood pressure response after administration of the respective antagonist. Doses of respective antagonists were as follows: hexamethonium bromide 3 mg/kg, i.v., atropine 2 mg/kg, i.v., propranolol 1 mg/kg, i.v., and diphenhydramine 7 mg/kg, i.v.
The highest hypotensive activity of D-I was observed with pentobarbital anaesthesia, a moderate activity was seen with α-chloralose and the least activity was noted with urethane.

In urethane anaesthetized rats, the depressor responses to D-I corresponded to approx. two-thirds of the animals under deep pentobarbital anesthesia. In spinal rats, the hypotensive effect of D-I was also observed though the hypotensive activity was much less potent than the activity in either conscious or anaesthetized normal rats. The hypotensive potency or its duration due to D-I was not affected by pretreatment with reserpine, while the pressor response to tyramine almost disappeared, as shown in Fig. 7. This disappearance of pressor response was not caused by tachyphylaxis.

Effects of various anatagonists on the hypotensive activity of D-I in urethane anaesthetized rats are illustrated in Fig. 8. While abolishment of hypertensive activity of 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) was observed in the hexamethonium pretreated rats, the hypotensive activity of D-I was not prevented in the same animal. The hypotensive effect of D-I given after treatment with hexamethonium was substantially greater than that before the treatment with the antagonist. As the effect of hexamethonium disappeared, the depressor responses returned to the level prior to the dosage of the antagonist.

**DISCUSSION**

The present paper is concerned with D-I, a hypotensive compound recently discovered in the bovine brain lipid fraction. The hypotensive activity of D-I was compared to that of acetylcholine as a standard and found to be compatible. D-I showed a uniform hypotensive effect, in the same order in all animals examined (Table 3, Fig. 4). Characteristics of D-I could be distinguished from those of PGF$_{2\alpha}$ (9, 10), lysophosphatidic acid (11), histamine, eledoisin (12) and physalaemin (13) which are species specific vasoactive substances. For example PGF$_{2\alpha}$ elicited depressor responses in cats or rabbits (9) and pressor responses in rats or dogs (10). The hypotensive potency of D-I varied by pretreatment with pentobarbital, α-chloralose and urethane (Figs. 5, 6). From the results obtained in examinations on spinal rats, we conclude that D-I does not exert a hypotensive action mainly via stimulation of the central nervous system. In findings obtained in reserpinized rats, the hypotensive effect of D-I does not appear to be related to catecholamines. As the hypotensive activity of D-I was unaffected by pretreatment with hexamethonium, this effect is apparently not directly related to ganglionic stimulating action.

With atropine or diphenhydramine, the hypotensive effect of D-I was unaffected, therefore, it may be presumed that the D-I effect was not due to contamination of acetylcholine nor histamine in the preparation (14), and that the hypotensive activity of D-I probably is not derived from stimulation on the cholinergic receptor or the histamine H$_1$ receptor. The hypotensive activity of D-I was not lost on the rats treated with propranolol, and the effect was not mediated through stimulation on the β-adrenergic receptors. Additionally it was confirmed that D-I showed no smooth muscle stimulating activity in isolated guinea pig ileum preparations (1). During perfusion of isolated rabbit ear preparations,
the relaxation of peripheral blood vessels was observed with administration of D-I (unpublished data). Thus, D-I does not elicit the depressor action via stimulation of the central and the autonomic nervous systems, rather a direct action on the peripheral blood vessels is suggested.

There are considerable data on bio-active lipids (14, 15). These data include hyper-or hypo-tensive substances, some of which remain chemically undefined and other compounds which although structurally defined have only low biological activities.

The PGs are in a separate category. From investigations on chemical characters, D-I is estimated to belong to the phospholipid group and has a lyso-type structure. Regarding cardiovascular activity of lyso-type phospholipids, the effect of lysolecithin on blood pressure of animals has not been entirely elucidated (16). Although several reports concerning renin inhibitory activity of lysophosphatidylethanolamines have been published, the hypotension was observed only in acute and chronic renal hypertensive rats (17–20). The potent cardiovascular activity of lysophosphatidic acid has been described in our own papers (11, 21, 22). Endoperoxide intermediates of PG biosynthesis have been isolated (23–27), and their pharmacological and biological effects examined (28, 29). However to our knowledge, there is no report regarding a phospholipid derivative of PGs, and the possibility that D-I is a phospholipid derivative of PGs was ruled out in our previous studies (1–3). Thus the highly purified hypotensive factor D-I may be a new naturally-occurring vaso-active substance.

Further pharmacological investigations and elucidation of the chemical structure of D-I are in progress.

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