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Effect of Alkaloids of Simaroubaceous Plants on the Local Blood Flow Rate

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Some alkaloids showing inhibitory effect on phosphodiestrase were tested for their effect on the rate of blood flow in intestine and stomach of rabbit. Canthin-6-one from *Picrasma quassioides* BENNET and β-carboline-1-propionic acid from *Ailanthus altissima* SWINGLE increased the rate of blood flow of intestine and stomach, while 4,5-dimethoxycanthin-6-one, 5-hydroxy-4-methoxycanthin-6-one and 1-methoxycarbonyl-β-carboline from *P. quassioides* increased the rate of intestinal blood flow only.

Keywords——blood flow; increase; alkaloids; canthin-6-one; 4,5-dimethoxycanthin-6-one; 5-hydroxy-4-methoxycanthin-6-one; β-carboline; 1-methoxycarbonyl-β-carboline; 1-ethyl-4,8-dimethoxy-β-carboline; β-carboline-1-propionic acid

In preceding papers,[1,2] we reported that some medicinal plants contain inhibitors of cyclic adenosine monophosphate (cAMP) phosphodiesterase (PDE). It is known that PDE inhibitors increase the volume of cAMP or cyclic guanidine monophosphate, by which they indirectly affect the action of hormones in cells according PDE inhibitors may be expected to be useful as: antianginal agent,[3] cerebral vasodilator,[4] antiasthmatic agent, antiallergic agent,[5] ureterolithiasis agent,[6] antiinflammatory agent[7] or antitumor agent,[8] and practically, papaverine, theophylline and caffeine are now used clinically medicine. From these facts, indicate that cAMP phosphodiesterase inhibitors found in the screening of natural products might have a variety of other pharmacological activities. Therefore, some PDE inhibitors may be expected to affect the rate of local blood flow in animals. A number of papers report local blood flow measurements by means of hydrogen clearance method described by Kety[9] and Aukland,[10] in brain,[11] cardiac muscle,[12] intestine,[13] skeletal muscle,[14] oviduct,[15] tooth,[16] stomach[17] and spinal cord.[18] However, no substances in crude drugs have been tested for their effect on the rate of local blood flow in animals. In this paper, plant and PDE inhibiting crude drugs to obtain biologically active compounds supposed to act on circulation were assayed for their effect on the intestinal blood flow rate of rabbit using hydrogen clearance method.

Of 40 samples tested and listed in TABLE I, 13 increased the rate of blood flow in intestine of rabbit by more than 20%. *Picrasma quassioides*, having more than 20% effect on the intestinal blood flow in the screening test was found to contain a number of PDE inhibiting alkaloids.[2] In the present experiments, those alkaloids having local blood flow increasing activity were identified as canthin-6-one and β-carboline alkaloids. In this paper, the relationship between the PDE inhibiting activity of those alkaloids and their effect on the local blood flow in intestine and stomach of rabbit will also be discussed.

Experimental

Crude drugs tested were as follows: Acori Calami Rhizoma, Ailanthi Cortex, Alismatis Rhizoma, Artemisiae Capillaris Flos, Astragali Radix, Carthami Flos, Chrysanthemi Flos, Clematidis Radix, Cnidii Rhizoma, Coptdis Rhizoma, Daphnis Genkwae Flos, Dichroae Radix, Forsythiae Fructus, Fritillariae Bulbus, Gardeniae Fructus, Gentianae Macrophyllae Radix, Hirudo, Lycii Cortex, Maydis Stigmata, Magnoliae Flos, Mori Cortex, Paeoniae Radix, Peucedani Radix, Picrasmae Lignum, Plantaginis Semen, Prunellae Spica, Puerariae Radix, Rehmanniae Radix, San-Chi Ginseng, Scrophulariae Radix, Scolopendra, Scutellariae Radix, Sophorae Flos, Tribuli

Fructus, Trapae Fructus, Uncariae Ramulus et Uncus, Visci Herba, Zanthoxyli Fructus, Zingiberis Rhizoma, Zizyphi Semen.

Ailanthi Cortex and Picrasmae Lignum were collected in the campus of Toho University and the rest were purchased from Uchida Pharmacy for Oriental Medicine (Tokyo).

Alkaloids——4,5-dimethoxycanthin-6-one,[19f] 5-hydroxy-4-methoxycanthin-6-one,[19f] 1-hydroxymethyl-β-carboline,[19f] 1-methoxycarbonyl-β-carboline,[19e] 1-ethyl-4,8-dimethoxy-β-carboline,[19c] 1-vinyl-4,8-dimethoxy-β-carboline[19d] from *Picrasma guassioides* and canthin-6-one,[19a] 1-methoxycanthin-6-one,[19a] canthin-6-one-3-oxide,[19a] 1-carbomoyl-β-carboline,[19e] 1-(1,2-dihydroxyethyl)-4-methoxy-β-carboline[19b] and β-carboline-1-propionic acid[19e] from *Ailanthus altissima* were tested for their effect on the rate of local blood flow of rabbits.

Materials and methods——Measurement of the rate of local blood flow was performed using a hydrogen gas monitor PHG-300 (M.T. Technical Institute, Tokyo). Electrocardiogram was recorded on Fukuda FCC-1 (Fukuda Co., Ltd., Tokyo). Albino rabbit, weighing between 2.5 and 3.5 kg were anesthetized with Nembutal-Na (Abbott) 40 mg/kg i.v., and laparotomy was performed. They were placed supine on a heating pad of about 36°C. Both ear veins were cannulated, one for injection of samples and the other for injection of dilute anesthesia during the experiment to maintain animals in a steady state.

Blood flow measurement——The sensors inserted in the intestine and stomach cortex together with a reference electrode of Ag-AgCl, placed under the abdomen, were connected to a hydrogen gas monitor, the output of which was recorded on a Hitachi dynograph. The wire for the sensor consisted of a bare-platinum wire of 0.2 mm diameter with a tip of length 1 mm coated with platinum black. The animals were forced to inhale 0.5 ml/min of 100% hydrogen gas for 12–15 sec. This amount of hydrogen gas does not saturate tissue with hydrogen gas; but still gives the same blood flow measurement results as when the tissues are saturated with hydrogen. The rate of blood flow was calculated from the clearance rate of hydrogen gas $F=0.693/T_{1/2}$ ml/min/g where $T_{1/2}$ is the time required for the clearance of 50% of the tissue hydrogen gas content. A hot aqueous extract from a medicinal plant was i.v. injected at a dose of 4.5 mg/kg 0.3 ml saline. Fractions from the extract from sap wood and heart wood of *Picrasma quassioides*, canthin-6-one and β-carboline derivatives were each administered i.v. at a dose of 0.3 mg/kg 0.05 ml DMSO. DMSO was used as a control. Measurement of the rate of blood flow was made 2, 10, 15, 20, 25 and 30 min, after the injection of samples. Recordings which failed to return to the base line were discarded. To avoid the erroneous effect of DMSO, or preceding samples, intervals of at least 1 hr were provided between one sample injection and the next sample injection and the experiments were started when stable base line was obtained. No significant effect was observed on heart rate during the experiments. These alkaloids are known not to increase blood pressure in rat.[20]

Extraction and separation——In the screening test, samples of medicinal plants (10 g) were extracted with water (100–150 ml) at 90–100°C for 6 hr and the extracts were concentrated, and lyophilized. Dried root sap wood chips and heart wood chips of *Picrasma quassioides* (30 g) were individually extracted with MeOH under reflux for 4 hr. The MeOH extract was partitioned between $CHCl_3$ and water. The $CHCl_3$ layer was shaken with 5% H_2SO_4 to give a basic fraction and a non-basic fraction. Each of the fractions was evaporated to dryness under reduced pressure, individually dissolved in DMSO and tested for their effect on the rate of local blood flow in duplicate.

4,5-Dimethoxycanthin-6-one, 5-hydroxy-4-methoxycanthin-6-one and 1-methoxycarbonyl-β-carboline——The main constituents of the basic fractions derived from *P. quassioides* were identified as 4,5-dimethoxycanthin-6-one (*Rf* 0.82), 5-hydroxy-4-methoxycanthin-6-one (*Rf* 0.79) and 1-methoxycarbonyl-β-carboline (*Rf* 0.84) on TLC plate [solvent: $CHCl_3$-MeOH(20:1)] by comparison with respective authentic samples.

Results and Discussion

Of 40 hot aqueous extracts of crude drugs tested 13 samples increased the rate of blood flow in intestine of rabbit by more than 20% as shown in TABLE I. Of the 13 effective samples, *P. quassioides*, containing PDE inhibition alkaloids was chosen for further studies on the relationship between PDE inhibiting activity, and the effect on the rate of local blood flow of rabbit. The alkaloid fractions derived from an extract from the sap wood of *P. quassioides* containing bitter principles, quassinoids and being used as a bitter stomachic in traditional oriental medicine, and from an extract from its heart wood were formed to be more effective in local blood flow test in rabbit than corresponding non-basic fractions, as shown in Fig. 1.

The basic fractions contained no quassinoides and their main constituents were identified as 4,5-dimethoxycanthin-6-one, 5-hydroxy-4-methoxycanthin-6-one and 1-methoxycarbonyl-β-carboline on TLC by comparison with respective authentic samples. TLC patterns of the basic-fractions of sap wood and of heart wood were quite similar. However, the content of alkaloids appeared to be much higher in the latter as demonstrated by the size of the spots on TLC plates, and some unidentified spots were found on the thin layer chromatogram of the basic-fraction from the sap wood when sprayed with Dragendorff

TABLE I. Effect of Crude Drugs on the Rate of Blood Flow in Intestine of Rabbit

Crude Drugs	Effect
Peucedani Radix (前胡)	+++
Scutellariae Radix (黄芩)	+++
Chrysanthemi Flos (菊花)	+++
Daphnis Genkwae Flos (芫花)	+++
Tribuli Fructus (蒺藜子)	+++
Zanthoxyli Fructus (山椒)	+++
Cnidii Rhizoma (川芎)	+++
Plantaginis Semen (車前子)	+++
Scolopendra (蜈松)	+++
Uncariae Ramulus et Uncus (釣藤鈎)	+++
Picrasmae Lignum (苦木)	++
San-Chi Ginseng (三七参)	++
Visci Herba (桑寄生)	++
Citri Leiocarpae Exocarpium (青皮)	+
Magnolia Flos (辛夷)	+
Prunellae Spica (夏枯草)	+
Trapae Fructus (菱実)	+
Zingiberis Phizoma (生姜)	+
Astragali Radix (黄耆)	−
Dichroae Radix (常山)	−
Gentianae Macrophyllae Radix (秦艽)	−
Paeoniae Radix (赤芍)	−
Scrophulariae Radix (玄参)	−
Puerariae Radix (葛根)	−
Alismatis Rhizoma (沢瀉)	−
Calami Rhizoma (菖蒲根)	−
Coptidis Rhizoma (黄蓮)	−
Ailanthi Cortex (神樹)	−
Lycii Radicis Cortex (地骨皮)	−
Mori Cortex (桑白皮)	−
Carthami Flos (紅花)	−
Sophorae Flos (槐花)	−
Gardeniae Fructus (山梔子)	−
Forsythiae Fructus (連翹)	−
Artemisiae Capillaris Herba (茵蔯蒿)	−
Fritillariae Bulbus (見母)	−
Hirudo (水蛭)	−
Lumbricus (地竜)	−
Maydis Stigmata (南蛮毛)	−
Zizyphi Spinosi Semen (酸棗仁)	−

Increase −: 0%, +: 10–20%, ++: 20–30%, +++: 30% and over.

reagent. Whether the higher activity shown by the sap wood in the intestinal blood flow test was due to this identified substance or to possible syurgestive or other effect of various components in sap wood is not known. Therefore, alkaloids previously isolated from *P. quassioides* were also tested for the rate of blood flow effect against intestine and stomach of rabbit.

Of 12 alkaloids tested, canthin-6-one, 4,5-dimethoxycanthin-6-one, 5-hydroxy-4-methoxycanthin-6-one, 1-methoxycarbonyl-β-carboline, 1-ethyl-4,8-dimethoxy-β-carboline and β-carboline-1-propionic acid (Fig. 2) were active in the local blood flow test and evaluation of their effect on the rate of blood flow in stomach and in intestine of rabbit is given in TABLE II. Figures 3 and 4 show time-course changes in their effect.

As shown in Figs. 3 and 4 and TABLE II, those six alkaloids increased intestinal blood flow rately more than 20% on average *i.e.* 4,5-methoxycanthin-6-one and 5-hydroxy-4-methoxycanthin-6-one induced the intestinal flow rate increase of about 20–30% and canthin-6-one of about 10%, whereas, only canthin-6-

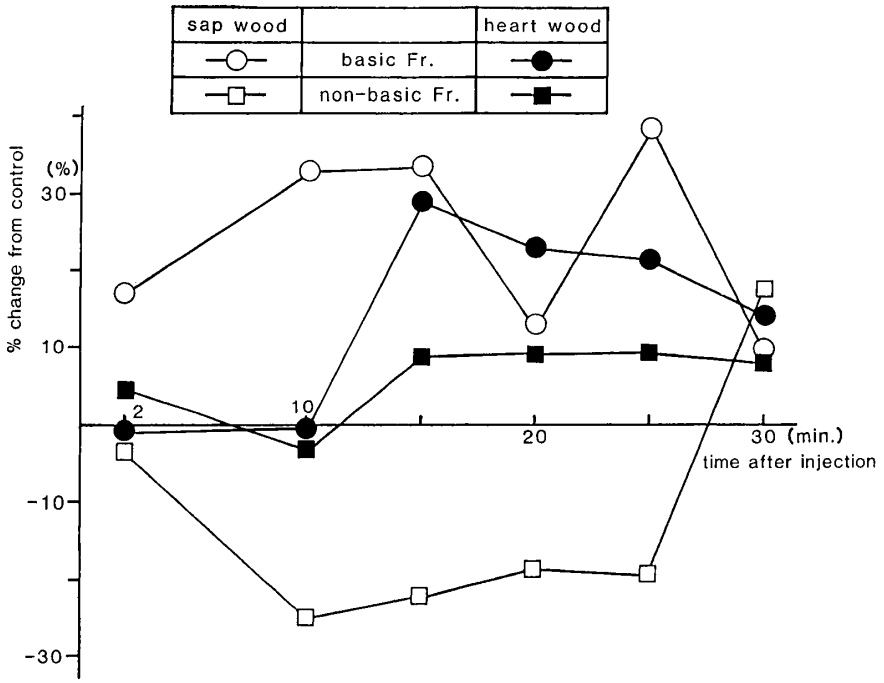

Fig. 1. Effect of Various Fractions of *Picrasma quassioides* on Intestinal Blood Flow Rate

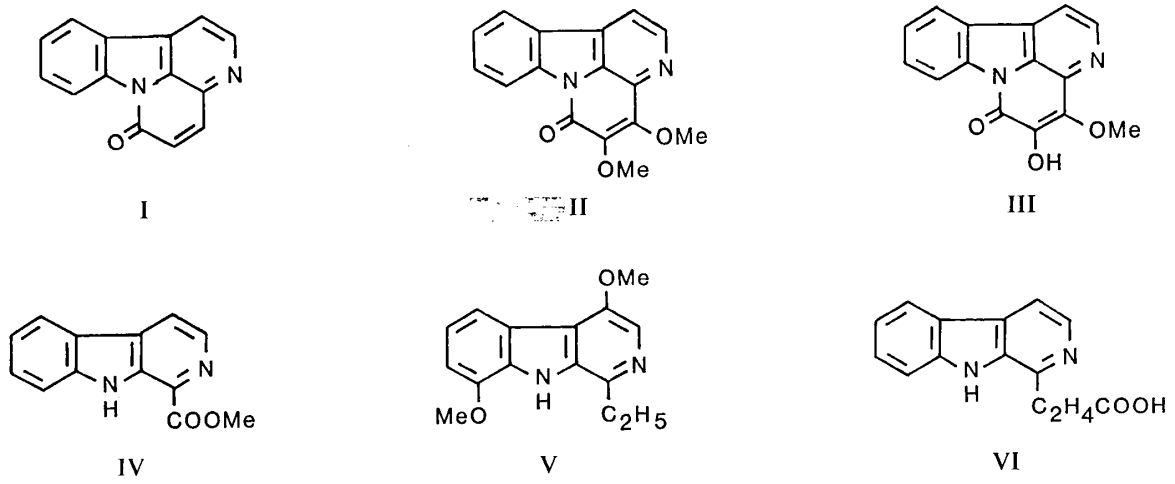

Fig. 2. Chemical Structures of Alkaloids

one and β-carboline-1-propionic acid from *Ailanthus altissima* SWINGLE increased the rate of stomachic blood flow by about 10% the latter inducting an increase in the intestinal blood flow rate of about 35 %, as shown in Fig. 4.

There was no significant difference between the IC_{50} value for PDE of papaverine and for example that of 1-methoxycarbonyl-β-carboline. However, papaverine increased intestinal blood flow rate by 10% at a low dose of 2 μg/kg while 1-methoxycarbonyl-β-carboline did at a dose of 0.3 mg/kg. The results show that substances affect the blood flow rate of different organs to a different extent. However, so far as the PDE-inhibiting alkaloids are concerned, as demonstrated in the present work, those having more porent PDE inhibiting activity tend to increase local blood flow rate more intensely.

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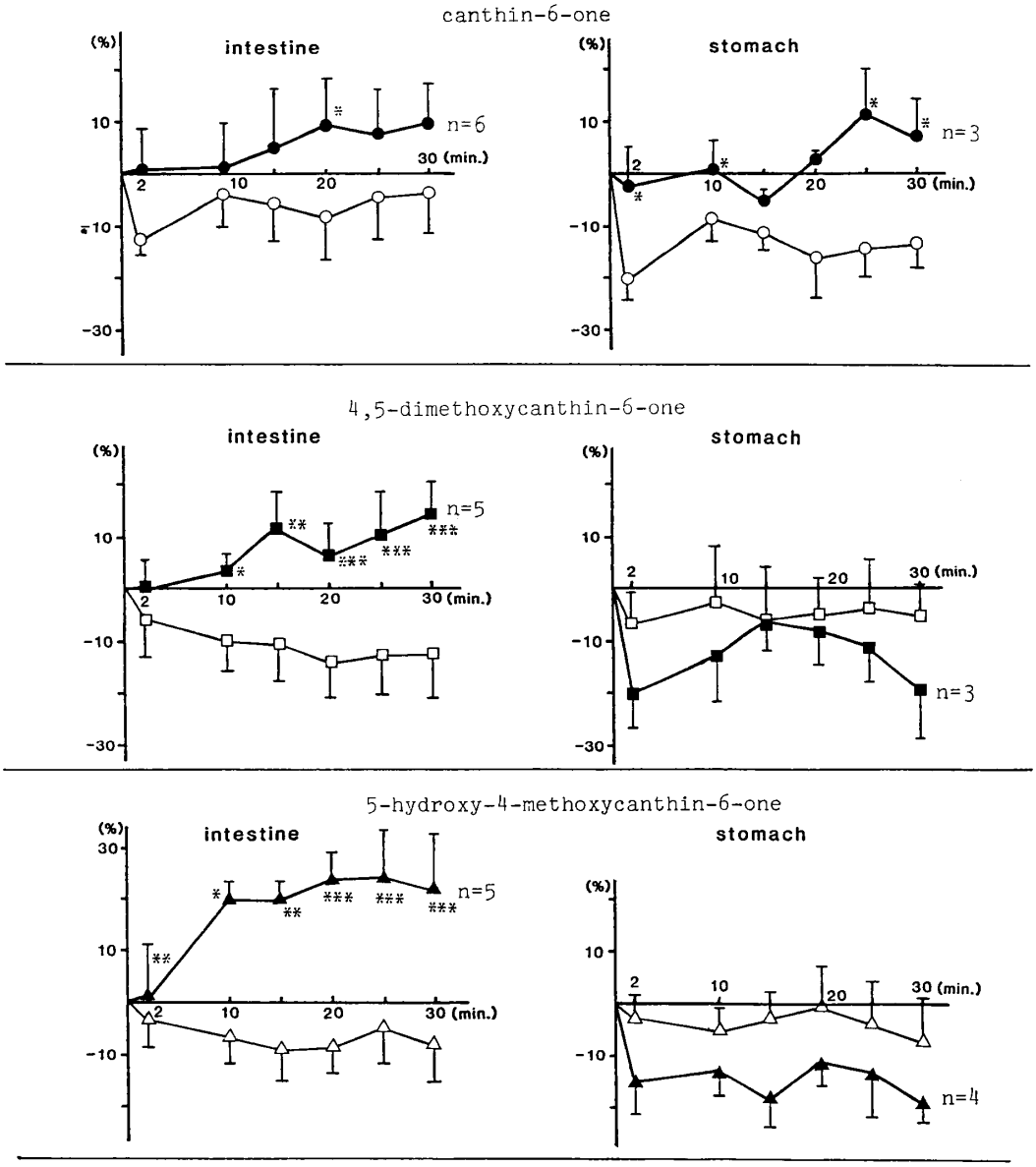

Fig. 3. Effects of Canthin-6-one Derivatives on Intestinal and Stomachic Blood Flow in Rabbits
Values are means ±SE. Significantly different from the control, $*p<0.05$, $**p<0.02$, $***p<0.01$. Open symbols: control (DMSO), Closed symbols: canthin-6-one derivatives.

TABLE II. Effect of Canthin-6-one and β-Carboline Derivatives on the Blood Flow Rate in Intestine and Stomach of Rabbit and on PDE Inhibition

Substance (0.3 mg/kg)	Effect on blood flow rate		PDE inhibition IC 50 ($\times 10^{-5}$ M)
	Intestine	Stomach	
	(%)	(%)	
Canthin-6-one (I)	+15*	+10	>100
4,5-Dimethoxycanthin-6-one (II)	+35	—**	1.4
5-Hydroxy-4-methoxycanthin-6-one (III)	+25	—	10.4
1-Methoxycarbonyl-β-carboline (IV)	+25	+ 5	3.6
1-Ethyl-4,8-dimethoxy-β-carboline (V)	+30	NT***	4.9
β-Carboline-1-propionic acid (VI)	+40	+20	16.5
Papaverine (2 μg/kg)	+10	—	3.0

* An average of the values during the period from 15 to 25 min after injection of sample.
** Decrease.
*** Not tested.

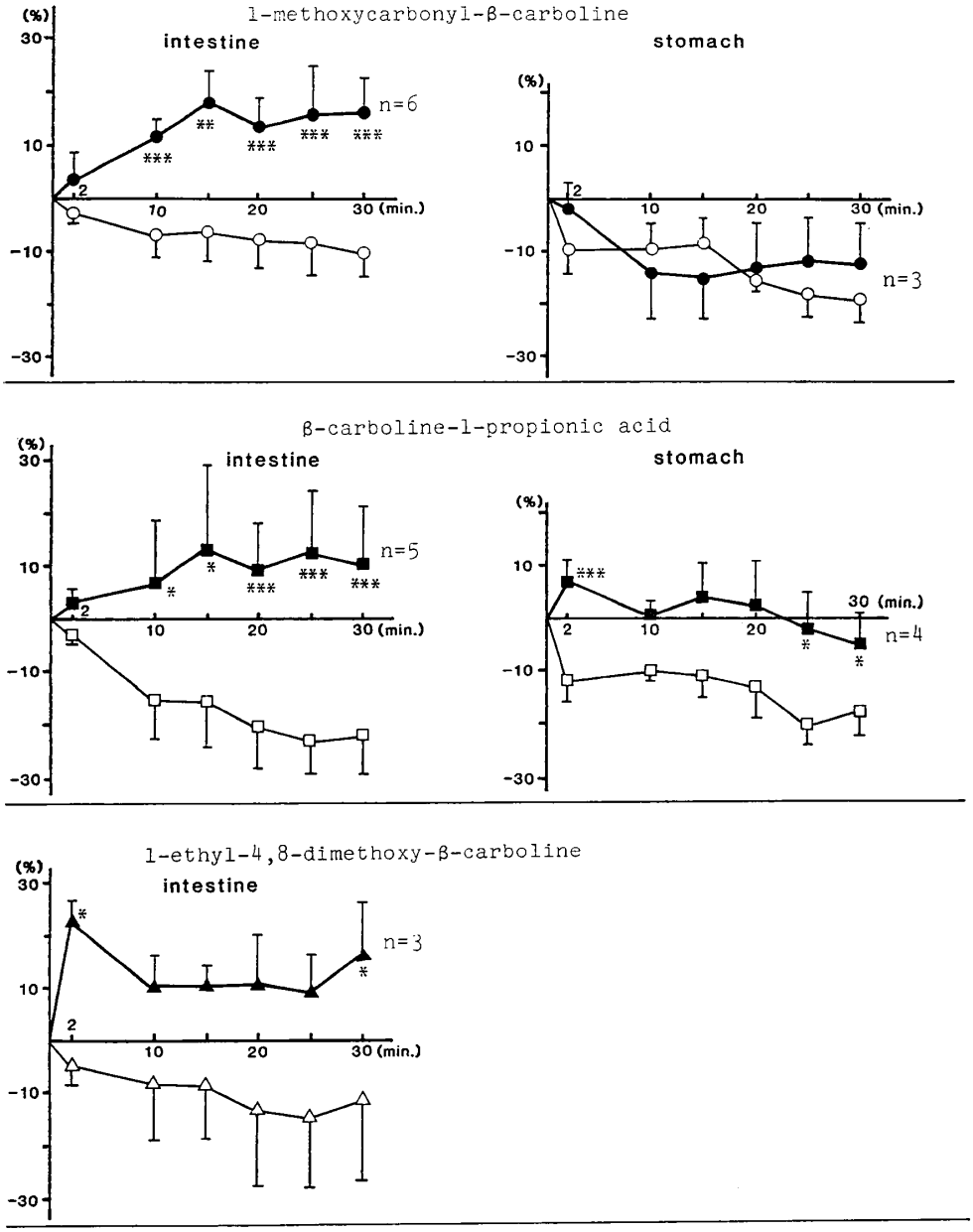

Fig. 4. Effects of β-carboline Derivatives on Intestinal and Stomachic Blood Flow in Rabbits
Values are means ±SE. Significantly different from the control, $*p<0.05$, $**p<0.02$, $***p<0.01$. Open symbols: control (DMSO), Closed symbols: β-carboline derivatives.

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