J. Pharm. Pharmacol. 1981, 33: 317–320
Communicated July 9, 1980

0022–3573/81/050317–04 $02.50/0


# Calcium antagonistic action of a coumarin isolated from "Qian-Hu", a Chinese traditional medicine

TOMOO KOZAWA, KIYOSHI SAKAI, MASAATSU UCHIDA*, TORU OKUYAMA, SHOJI SHIBATA†, *Department of Molecular Pharmacology and Department of Pharmacognosy and Phytochemistry,† Meiji College of Pharmacy, 1-35-23 Nozawa, Setagayaku, Tokyo 154 Japan*

Calcium antagonists are useful in therapy of cardiovascular diseases and are also useful in studies of excitation-contraction coupling and stimulus-secretion coupling (Fleckenstein 1977). Thus it is now important to find prototypes of calcium antagonists for pharmacological use. "Qian-Hu" the roots of *Peucedanum praeruptorum* Dunn (Umbelliferae) is a traditional Chinese medicine used in alimentary and bronchial disorders and chest pain, presumably including angina pectoris, therefore, we examined the effects of its extracts on smooth muscle.

*Methods and materials*

Male guinea-pigs, 200–400 g, were killed by cervical fracture and exsanguination. Strips of ileum or taenia coli were suspended in a 30 ml organ bath at 30 °C and bubbled with air. Responses were recorded isotonically. The test compound was added 5 min before recording cumulative contractile responses. The antagonistic actions to acetylcholine and histamine were tested on ileum in Locke-Ringer solution (mM: NaCl 154, KCl 5·63, $CaCl_2$ 2·16, $MgCl_2$ 2·10, $NaHCO_3$ 5·95, glucose 5·55). Taenia coli was used to test the relaxing effects on $K^+$-depolarized muscle, it was first induced to contract in Locke-Ringer solution by adding KCl (40 mM final concn) and the contraction height taken as 100%. The muscle was then washed with $Ca^{2+}$-free Locke-Ringer solution containing 3 mM EGTA (glycol ether diaminetetraacetic acid, Dojin Laboratories). When the muscle had relaxed to basal level, it was suspended in $Ca^{2+}$-free KCl-Locke-Ringer solution (mM: KCl 160, $MgCl_2$ 2·10, $KHCO_3$ 5·95, glucose 5·55) and $CaCl_2$ was added cumulatively.

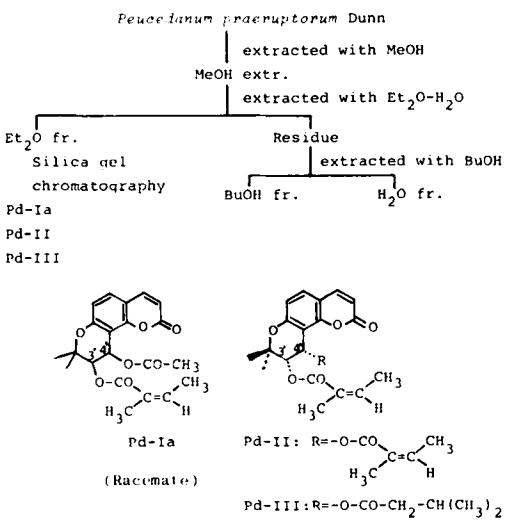


FIG. 1. Fractionation of components of "Qian-Hu" and the structures of the coumarins isolated.

The uptake of $^{45}Ca^{2+}$ by smooth muscle was measured as described by Tomiyama et al (1973). The taenia coli strip was attached to a glass hook and placed in $Ca^{2+}$-free Locke-Ringer solution containing 3 mM EGTA at 30 °C for 30 min aerated with 95% $O_2$ and 5% $CO_2$. Then the medium was replaced by aerated $Ca^{2+}$-free KCl-Locke-Ringer solution at 30 °C. The drug was added and 5 min later $CaCl_2$ at a final concentration of 1 mM containing $^{45}Ca^{2+}$ (1 $\mu$Ci ml$^{-1}$) was added. Samples of muscle were transferred 3, 6 and 9 min later to Locke-Ringer solution with 10 mM $LaCl_3$ and with an equimolar concentration of HEPES (*N*-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid, Dojin Laboratories) in place of $NaHCO_3$. The solution was kept at 4 °C and bubbled with 100% $O_2$. After 60 min the tissue was removed, blotted on a filter paper and quickly weighed. It was then digested with Soluene 350 (Packard Instrument Co.) and its radioactivity counted.

The tissue concentration of cyclic (c)AMP was estimated by the method of Gilman (1970). Two pieces of taenia coli were removed from one caecum and each was suspended in a 30 ml organ bath. One piece was relaxed by the test drug and then both were rapidly frozen in liquid nitrogen, homogenized in a glass homogenizer in 2 ml of ice cold trichloroacetic acid (6% w/v) and cAMP estimated as described by Inatomi et al (1974).

Table 1. Cyclic AMP content of taenia coil in the presence and absence of Pd-Ia.

| Drug | cyclic AMP level (mean ± s.e.) p mol mg$^{-1}$ wet weight | % change | number of experiments | P value |
|---|---|---|---|---|
| control | 0·41 ± 0·03 | 100 | 14 | |
| Pd-Ia (1 × 10$^{-5}$ g ml$^{-1}$) | 0·50 ± 0·04 | 124 | 12 | >0·05 |
| control | 0·44 ± 0·03 | 100 | 14 | |
| Pd-Ia (1 × 10$^{-4}$ g ml$^{-1}$) | 0·49 ± 0·04 | 112 | 13 | >0·05 |

* Correspondence.

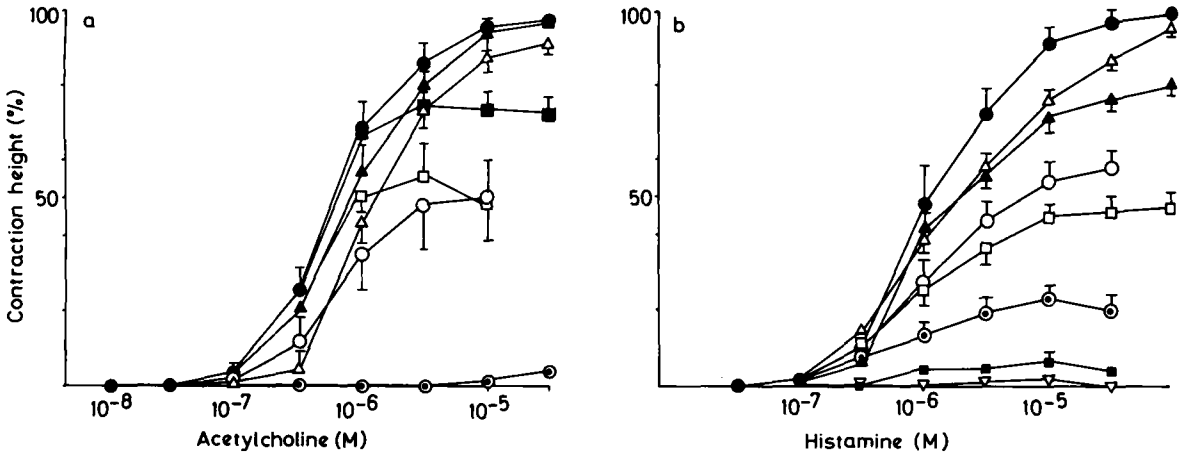


FIG. 2. Effects of "Qian-Hu" extracts on contractile responses of guinea-pig ileal smooth muscle induced by acetylcholine (a) and histamine (b). Points are means of 6 experiments. Vertical bars show s.e. (a) ●: control, ▲: $H_2O$ fr. $1 \times 10^{-4}$, △: BuOH fr. $1 \times 10^{-4}$, ■: $Et_2O$ fr. $1 \times 10^{-5}$, □: $Et_2O$ fr. $3 \times 10^{-5}$, ○: $Et_2O$ fr. $1 \times 10^{-4}$, ○: MeOH ex. $1 \times 10^{-4}$ (g ml$^{-1}$) (b) ●: control, △: $H_2O$ fr. $1 \times 10^{-4}$, ▲: BuOH fr. $1 \times 10^{-4}$, ○: $Et_2O$ fr. $1 \times 10^{-5}$, ⊙: $Et_2O$ fr. $3 \times 10^{-5}$, ▽: $Et_2O$ fr. $1 \times 10^{-4}$, □: MeOH ex. $3 \times 10^{-5}$, ■: MeOH ex. $1 \times 10^{-4}$ (g ml$^{-1}$).

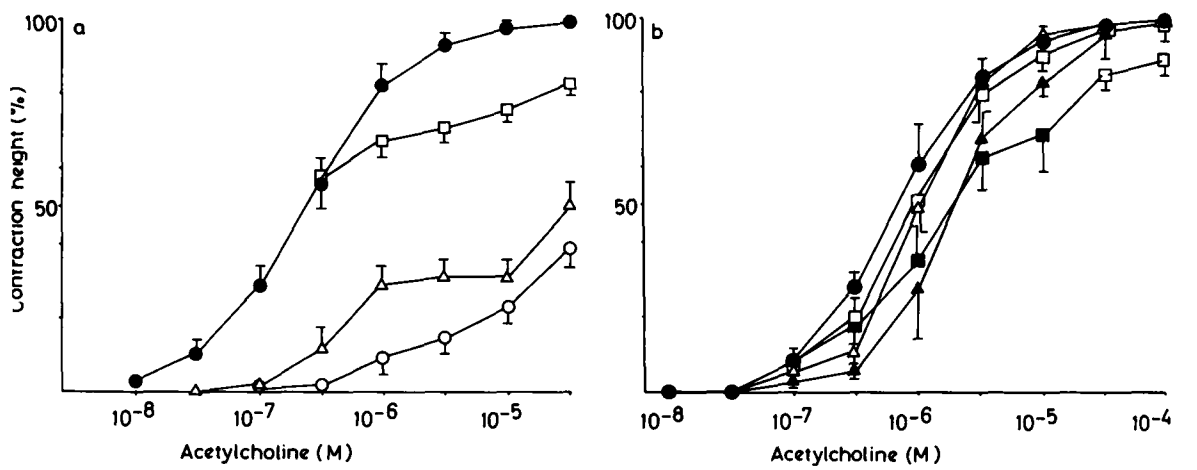


FIG. 3. Effects of coumarins isolated from "Qian-Hu" on contractile responses of guinea-pig ileal smooth muscle induced by acetylcholine. Points are means of 6 experiments. Vertical bars show s.e. (a) ●: control, □: Pd-Ia $3 \times 10^{-6}$, △: Pd-Ia $1 \times 10^{-5}$, ○: Pd-Ia $3 \times 10^{-5}$ (g ml$^{-1}$) (b) ●: control, □: Pd-II $1 \times 10^{-5}$, ■: Pd-II $3 \times 10^{-5}$, △: Pd-III $1 \times 10^{-5}$, ▲: Pd-III $3 \times 10^{-5}$ (g ml$^{-1}$).

Extracts of "Qian-Hu" were prepared from the roots of *Peucedanum praeruptorum* Dunn as described by Okuyama & Shibata (1981).

The roots were extracted twice with MeOH under reflux for 8 h. The MeOH extract was then concentrated under vacuum and the residue dissolved in water and fractionated into an ether-soluble fraction ($Et_2O$ fraction) and a water-soluble fraction, the latter divided into a BuOH-soluble fraction (BuOH fraction) and a water-soluble fraction ($H_2O$ fraction). $Et_2O$ fraction was applied to a silica gel column and coumarins were eluted with n-hexane-ethyl acetate (Fig. 1). Test compounds were dispersed with Tween 80 (0·01%), which had negligible effects in the systems tested.

*Results*

The $Et_2O$ fraction of "Qian-Hu" was the most effective in antagonizing contraction of guinea-pig ileum induced by acetylcholine and histamine. Its mode of action was non-competitive Fig 1. Among the coumarins, Pd-Ia [=(±)-praeruptorin A (Chen et al 1979)] was the most effective in antagonizing the action of acetylcholine non-competitively on small intestine (Fig. 2). Pd-Ia suppressed contraction of $K^+$-depolarized

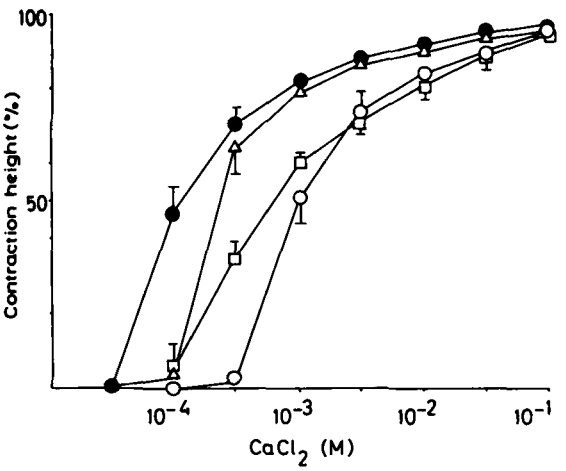


FIG. 4. Effects of Pd-Ia, papverine and the ether fraction on the cumulative dose-response curve of $CaCl_2$ tested on the $K^+$-depolarized taenia coli of guinea-pig. Points are means of 6 experiments. Vertical bars show s.e. ●: control, △: $Et_2O$ fr. $3 \times 10^{-6}$, □: Papaverine $3 \times 10^{-6}$, ○: Pd-Ia $3 \times 10^{-6}$ (g $ml^{-1}$).

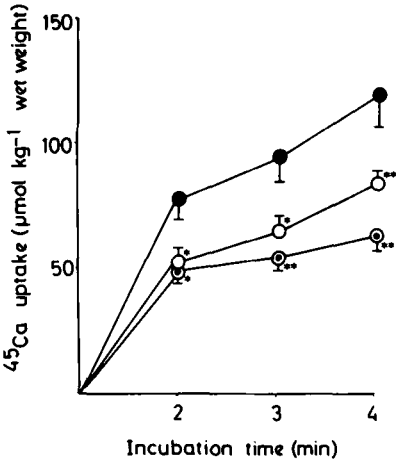


FIG. 6. Uptake of $^{45}Ca$ by taenia coli of guinea-pig in the presence and absence of Pd-Ia. Points are means (n = number of experiments) and vertical bars show s.e. $*P < 0.05$ $**P < 0.01$; significance from the control. ●: control (n = 10), ○: Pd-Ia ($1 \times 10^{-5}$ g $ml^{-1}$) (n = 6), ⊙: Pd-Ia ($3 \times 10^{-5}$ g $ml^{-1}$) (n = 4).

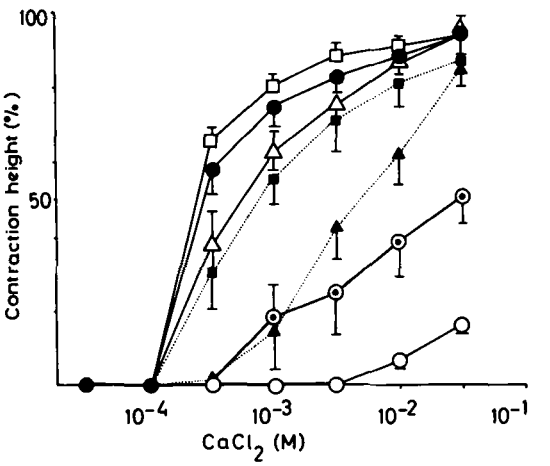


FIG. 5. Effects of coumarins isolated from "Qian-Hu", compound D-600 and khellin on the cumulative dose-response curve of $CaCl_2$ tested on $K^+$-depolarized taenia coli of guinea-pig. Points are means of 4 experiments. Vertical bars show s.e. ●: control, △: Pd-II $3 \times 10^{-5}$, □: Pd-III $3 \times 10^{-5}$, ■: khellin $3 \times 10^{-5}$, ▲: D-600 $5 \times 10^{-8}$, ○: Pd-Ia $1 \times 10^{-5}$, ⊙: Pd-Ia $3 \times 10^{-5}$ (g $ml^{-1}$).

smooth muscle induced by influx of extracellular $Ca^{2+}$ into guinea pig taenia coli ($pA_2$ for $Ca^{2+}$ 6·14) (Fig. 3) but Pd-II [=(+)-anomalin (Hata et al 1968)] and Pd-III [(+-3′(*S*)-angeroyloxy-4′(*S*)-ivovaleryloxy-3′,4′-dihydro-seselin Okuyama & Shibata (1981)] did not affect the contraction significantly. Pd-Ia inhibited the entry of $^{45}Ca^{2+}$ into smooth muscle cells (Fig. 4). Pd-Ia had a negligible effect on the tissue level of cAMP (Table 1).

*Discussion*

Pd-Ia caused non-competitive relaxation of ileum contracted by acetylcholine. As it inhibited $K^+$-induced contraction of taenia coli it is a musculotropic antispasmodic. Since the $K^+$-induced contraction the intestinal smooth muscle is thought to depend on increased influx of $Ca^{2+}$ across the membrane, compounds that inhibit the contraction may be assumed to inhibit $Ca^{2+}$ influx. This postulation was substantiated in studies on $^{45}Ca^{2+}$ influx into muscle cells under conditions where the participation of extracellular $Ca^{2+}$ spaces (van Breeman et al 1972) and efflux of $^{45}Ca^{2+}$ from the cells (Deth 1978) were eliminated. Pd-Ia inhibited the influx of $^{45}Ca^{2+}$ into the smooth muscle cells. Papaverine which has a stronger antispasmodic action than Pd-Ia is less inhibitory of $Ca^{2+}$ influx ($pA_2$ for $Ca^{2+}$ 5·88, Fig. 4). As calcium antagonists are effective in the therapy of angina it can be seen why "Qian-Hu" has been used for relieving anginal chest pain.

We used $Ca^{2+}$-induced contraction of $K^+$-depolarized taenia coli to avoid problems associated with arterial smooth muscle (Uchida et al 1978). In support of our choice of system is that nifedipine, a potent calcium antagonist currently used in the treatment of angina, does not cause dilatation of the artery under normal physiological conditions but is effective in inhibiting $Ca^{2+}$-induced contraction of $K^+$-depolarized taenia coli, suggesting that a drug with activity on $K^+$-depolarized smooth muscle of the taenia coli should be effective in relief of angina.

The calcium antagonistic activity of Pd-Ia is stronger than that of etafenone ($pA_2$ 5·95), a moderate coronary dilator, and weaker than that of prenylamine ($pA_2$ 6·44),

a calcium antagonist with catecholamine-depleting activity. Pd-Ia itself, which has a lower $pA_2$, may be of little value as a therapeutic drug for angina pectoris after development of several potent calcium antagonists effective in relief of angina such as diltiazem ($pA_2$ 6·90), verapamil ($pA_2$ 7·36) and nifedipine ($pA_2$ 9·43) [$pA_2$ values according to Imai (1980)]. But its effect is interesting from a comparative view point because Pd-II and Pd-III, which are structurally similar, had no effect in blocking $Ca^{2+}$ influx.

REFERENCES

Chen, Z. X., Huang, B. S., She, Q. L., Zeng, G. F. (1979) Acta Pharmaceutica Sinica 14: 486–496

Deth, R. C. (1978) Am. J. Physiol. 234: C139–145

Fleckenstein, A. (1977) Ann. Rev. Pharmacol. 17: 149–166

Gilman, A. G. (1970) Proc. Natl. Acad. Sci. 67: 305–312

Hata, K., Kozawa, M., Ikeshiro, Y., Yen, K. Y. (1968) Yakugaku Zasshi 88: 513–520 (in Japanese)

Imai, S. (1980) TIPS 1: 87–89

Inatomi, N., Takayanagi, I., Uchida, M., Takagi, K. (1974) Eur. J. Pharmacol. 26: 73–76

Okuyama, T., Shibata, S. (1981) Planta Medica 41: in the press

Tomiyama, A., Takayanagi, I., Takagi, K. (1973) J. Pharm. Pharmacol. 25: 65–68

Uchida, M., Yoshimoto, N. M., Harumi, K., Murao, S. (1978) Jpn. Heart J. 19: 281–296

van Breeman, C., Farinas, B. R., Gerba, P., McNaughton, E. D. (1972) Circ. Res. 30: 44–52

J. Pharm. Pharmacol. 1981, 33: 320–322
Communicated December 15, 1980

0022–3573/81/050320–03 $02·50/0


# Structure-activity relationships among hallucinogenic tryptamine derivatives evaluated by schedule-controlled behaviour

R. ADRON HARRIS*, ENNO HOMFELD**, E. CAMPAIGNE**, *The Harry S. Truman Memorial Veterans Hospital and *the Department of Pharmacology, University of Missouri Medical Center, Columbia, Missouri, 65212, and **the Chemistry Laboratories, Indiana University, Bloomington, Indiana, 67401, U.S.A.*

A number of benzo[*b*]thiophenes which are sulphur isosteres of *NN*-dimethyltryptamine (DMT,I) and related hallucinogens have been synthesized (Campaigne et al 1968; Campaigne & Dinner 1970; Campaigne et al 1970; Campaigne & Rogers 1973) and the 1-methylindole and naphthalene isosteres of DMT are also available (Fig. 1). As the effect on animal behaviour of alterations of the indole moiety of such compounds has received little attention, we used the method of Harris (1980) to examine the behavioural effects of DMT, DMT derivatives, and DMT isosteres on schedule-controlled behaviour of the rat. With this system, we have previously found that the potency of hallucinogenic drugs correlates with their potency in producing subjective effects in man (Harris et al 1977, 1978).

Nine rats were used. The apparatus and procedure (lever pressing under a fixed-interval 5-min schedule of food presentation) were essentially as described by Harris (1980), except that animals were divided into two groups. For one group, sessions ended after 11 intervals or 70 min, whichever occurred first. Due to the short duration of action of some of the drugs, data were recorded after the first 30 min of each session. For the other group, sessions ended after 300 min and data were recorded every 30 min throughout the sessions. Sessions were conducted five days each week.

*NN*-Dimethyltryptamine and 5-methoxy-*NN*-dimethyltryptamine were purchased from Sigma Chemical Co. (St Louis, Mo.); psilocin was kindly provided by the National Institute on Drug Abuse (Rockville, Md.). The remaining compounds (Fig. 1) were synthesized (Campaigne et al 1968; Campaigne & Dinner 1970; Campaigne & Rogers 1973). Drugs were dissolved in 0·9% NaCl and injected intraperitoneally 1 ml $kg^{-1}$ 2 min before the beginning of the behavioural sessions. Dosages were expressed as the free base for DMT, 5-MeO-DMT, psilocin and compound IX, for the remaining compounds as the hydrochloride. Dose-response curves were determined by testing three to four doses of each drug in three to five rats. Each dose was administered twice to each rat. Drug injections were usually on Tuesdays and Fridays, and were separated by at least 72 h. Control data were obtained on Thursdays. The dose of each drug required to reduce responding to 50% of the control rate (ED50) was estimated by plotting percent of control responding versus log dose (Harris et al 1978; Harris 1980). The 95% confidence limits of the ED50 values were determined by the parallel line assay of graded responses (Goldstein 1964).

Control rates of responding were initially low and increased toward the end of each interval, in agreement with Harris et al (1978) and Harris (1980). Average rates of responding were stable under control conditions.

All drugs decreased the average rate of responding during the first 30 min. Their potencies were estimated from dose-response curves (Table 1). The tryptamine derivatives, DMT(I), psilocin (VIII), and 5-MeO-DMT (XII) were the most potent; their sulphur (benzo[*b*]-thiopene) analogues were less so, that of compound (XIII) being about 15 times weaker than 5-MeO-DMT. Addition of a methyl group to the indole nitrogen (1-methylindole) also reduced the potency of DMT

* Correspondence.