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IN VITRO STUDY OF THE ANTICHOLINERGIC AND ANTIHISTAMINIC ACTIVITIES OF PROTOPINE AND SOME DERIVATIVES

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Protopine (fumarine, macleyine) is one of the more commonly available alkaloids, being widely distributed among members of the plant families Berberidaceae, Ranunculaceae, Rutaceae, Fumariaceae, and Papaveraceae (1). Protopine demonstrated antiallergic activity on guinea pigs and rabbits in vivo (2). It has an inhibiting action on guinea-pig intestine (3). On rabbit and rat isolated intestinal smooth muscle, some antispasmodic activity of protopine has been reported (4). As an experimental antiarrhythmic agent, it is 2–3 times more potent than quinidine and procainamide in rats (5). A strong antibacterial effect on staphylococci has been observed (6). Recently, protopine was reported to enhance the γ-aminobutyric acid receptor binding to rat brain synaptic membrane receptors (7). We studied the anticholinergic and antihistaminic properties of protopine and some derivatives on the isolated guinea-pig ileum.

Cumulative dose-response curves for acetylcholine were displaced to the right by protopine in a parallel manner (characteristic for competitive antagonism). The effect was qualitatively comparable to atropine, although higher concentrations of protopine ($>10^{-5}$ M) decreased the maximal contractions of acetylcholine (Figure 1). The latter effect indicates some noncompetitive antagonistic activity for protopine. The pA_2 value for protopine was 5.87 ± 0.21 (SE) ($N = 15$). The slope included -1 (-1.00 ± 0.22), which can be considered as a proof of competitive antagonism. Atropine had a pA_2 of 8.69 ± 0.23 ($n = 12$), with a slope of -1.09 ± 0.15. In consequence, atropine was revealed to be about 660 times more potent than protopine as an anticholinergic agent.

There was a slight increase of activity when longer preincubation times for protopine were used: pA_2 values were 5.88 (5.68–5.89) after 10 min incubation and 6.14 (5.92–6.36) after 20 min incubation ($n = 2$ for each incubation time). There was no increase of the noncompetitive effect in these experiments. The protopine activity could easily be washed out.

The styrene derivative of protopine (1) had some slight contractile activity by itself, and a noncompetitive effect toward acetylcholine ($pD'_2 = 4.18 \pm 0.34$, $n = 4$). Under the same experimental conditions, papaverine was 10 times more potent ($pD'_2 = 5.08 \pm 0.18$) (8).

Incubation studies using histamine as the agonist indicated that protopine does not have true antihistaminic properties.

Other chemical derivatives of protopine (protopine *N*-oxide and its pyrolysis product dibenzoxazacyclo-undecine) (1) did not have competitive antagonistic properties toward acetylcholine. They showed variable contractions by themselves without exceeding 20% of the maximal contractions in the concentration range used (2.8×10^{-5} to 2.8×10^{-6} M).

In conclusion, protopine can be considered as a weak anticholinergic alkaloid. Occurrence of protopine in different plant families might be responsible for reported spasmolytic properties of several medicinal plants.

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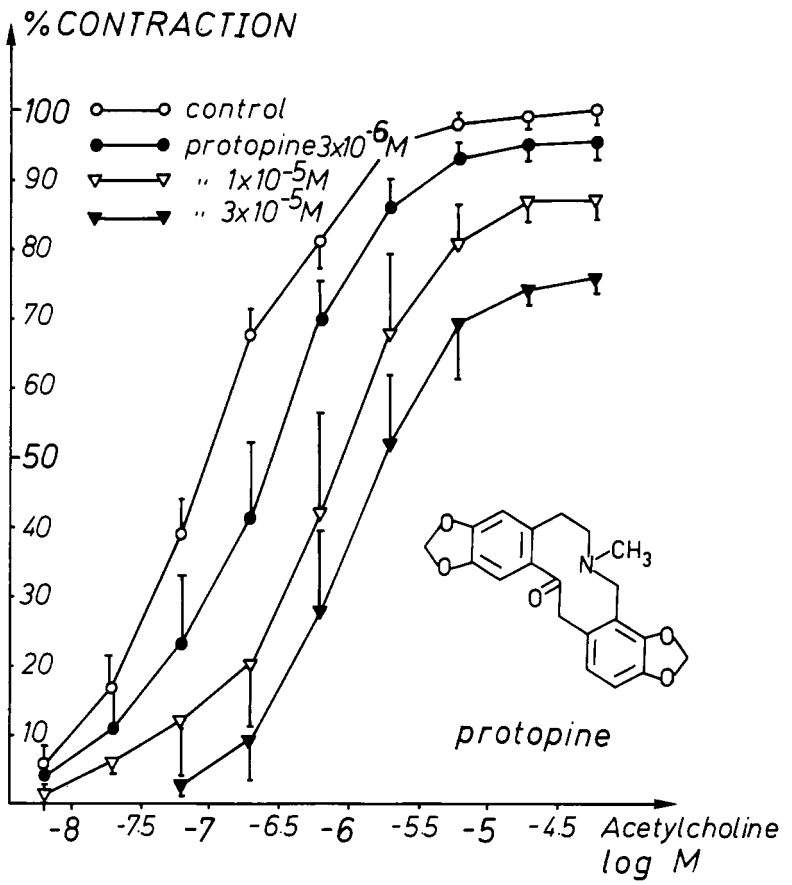

FIGURE 1. Cumulative concentration-response curves for acetylcholine on the guinea-pig ileum after 5 min preincubation with protopine. Each value represents the mean ± SEM of at least 4 experiments.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—In vitro isolated organ bath techniques were used that have been reported elsewhere (9). Ileum strips from guinea pigs (male and female, 300–400 g) were put in isolated organ baths filled with Tyrode solution and bubbled with an O_2-CO_2 (95:5) mixture. Cumulative concentrations of acetylcholine or histamine were given at 10 min intervals. Antagonists were preincubated for 5 min prior to the cumulative agonist concentrations. pA_2 values were calculated according to Tallarida and Murray (10).

CHEMICALS.—Protopine and its synthetized derivatives were supplied by B. Gözler. Stock solutions were prepared in absolute EtOH (Merck) and diluted with distilled H_2O. Acetylcholine·HCl (Merck) and histamine·diHCl (Pharmachemic) were dissolved in distilled H_2O.

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