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N-(3-Benzylthio-2,6-dichlorophenyl)anthramyl acid (ASD 30): a non-competitive antagonist of bradykinin

SIR,—In 1966 methixene was reported to be a non-competitive antagonist of bradykinin (van Riezen, 1966). Recently Drs M. Taeschler and A. Fanchamps informed us that ASD 30 was a selective bradykinin antagonist. We have now examined the mechanism of action of this compound by the method used for methixene.

Guinea-pig ileum was bathed in a 10 ml bath with a Tyrode solution saturated with a mixture of oxygen 95% and carbon dioxide 5% at 37°. Two cumulative

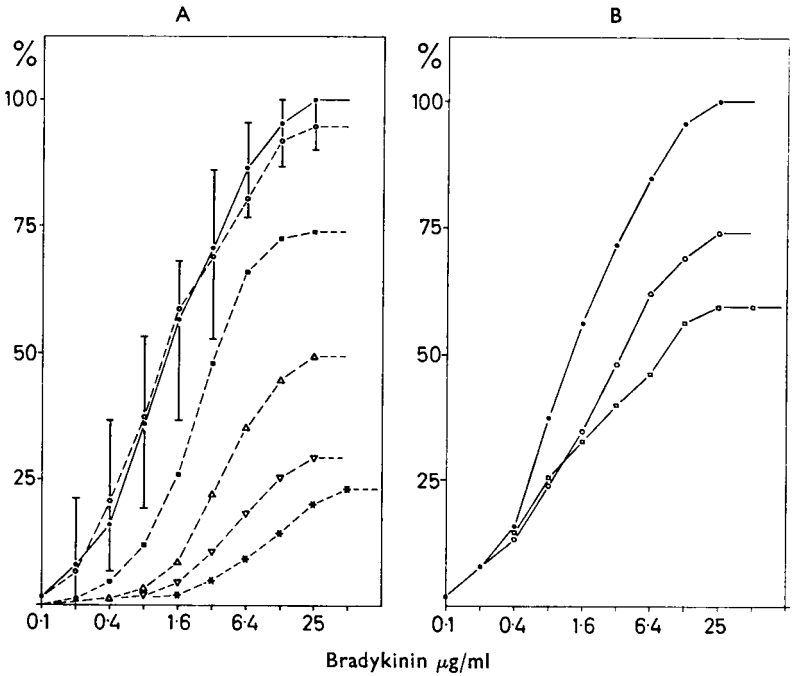

FIG. 1A. Cumulative dose response curves of guinea-pig ileum in Tyrode solution saturated with oxygen 95% and carbon dioxide 5% at 37° to bradykinin. With ASD 30 (mole/ml): ○- - - - -○ 1.10^{-6} (4); □- - - - -□ 3.10^{-6} (6); △- - - - -△ 1.10^{-5} (6); ▽- - - - -▽ 2.10^{-5} (2); *- - - - -* 5.10^{-5} (2). ●——● Control (20) 95% confidence limits. In parentheses: number of individual curves from which the curve is calculated.

B. In this experiment, the period of 30 min washing was followed by a second because the bradykinin curve was still below its control value. After this second wash the 1 hr curve ○——○ (2.10^{-5}mole/ml) was made. Then the preparation was again incubated with ASD 30 (2.10^{-5}mole/ml) for 20 min and the procedure repeated: curve □——□.

dose response curves with bradykinin were made and if these curves differed by less than 10% the experiment was begun. ASD 30 was dissolved by heating the required amount for one day's experimentation in 1–2 ml of N NaOH in a boiling water bath. This solution was then diluted with Tyrode to the required concentrations. The guinea-pig ileum was incubated with ASD 30 added to the medium to a final concentration of 1.10^{-6}, 3.10^{-6}, 1.10^{-5}, 2.10^{-5} or 5.10^{-5} mole/ml for 20 min, then cumulative dose response curves to bradykinin in the presence of the inhibitor were made. The guinea-pig ileum was then washed several times with fresh Tyrode solution during 30 min and a control cumulative dose response curve with bradykinin was made. The curves were readily reproducible. The same preparation was often used for more interaction curves whereas the same final inhibitor concentrations were tested in several preparations of guinea-pig ileum. A given dose was considered maximal if a two-fold higher dose did not induce an increased contraction of the preparation.

In Fig. 1A, cumulative dose-response curves to bradykinin (mean of 20 experiments) and of bradykinin in the presence of different concentrations of ASD 30 are shown. In the presence of 1.10^{-6}mole/ml ASD 30 no antagonism was seen. However, 3.10^{-6}mole/ml ASD 30 decreased the effect of bradykinin almost proportionately for each dose. This effect was more pronounced with the higher dose of inhibitor. The slope of the curves seemed to be dose-dependent, indicating a noncompetitive inhibitory mechanism. During the experiment with the doses of 2.10^{-5}mole/ml ASD 30 and higher, it became evident that the effect of ASD 30 was not completely reversible.

Fig. 1B shows the effect of 20 min incubation of guinea-pig ileum in Tyrode solution containing 2.10^{-5} mole/ml ASD 30. After a bradykinin response curve was made in the presence of the inhibitor, the preparation was washed several times during 1 hr with Tyrode solution without the inhibitor and a control bradykinin curve made. The sequence was then repeated a second time. It can be seen that even after 1 hr of washing, the bradykinin curve is only about 75% of the control. After another incubation period with the same concentration of ASD 30 the maximal response to bradykinin was 60%, after 1 hr of washing.

Thus ASD 30 seems to be a non-competitive bradykinin antagonist when tested on the guinea-pig ileum, the effect being only partially reversible in the higher dose ranges. The search for bradykinin antagonists has not yet uncovered a competitive bradykinin antagonist. Our method, being simple and rapid, might be useful in this search. The activity of bradykinin on the guinea-pig ileum does not seem to be related to the postulated role of the kinin as mediator of inflammatory and similar pathological conditions, and results from the ileum of the guinea-pig should be extrapolated with utmost reserve to other tissues.

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