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POTENTIATION OF THE RESPONSE TO HISTAMINE BY PICOLYLAMINES

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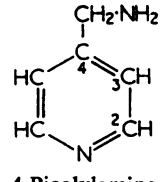
The response of the isolated ileum of the guinea-pig to histamine is potentiated in the presence of 2-, 3- or 4-picolylamine. These compounds have been found to inhibit the histaminase and/or "diamine oxidase" of pig kidney. The three corresponding picolylmethylamines did not potentiate the response of the ileum to histamine; they were without significant affinity for the pig kidney oxidase. It is suggested that the potentiating action of the three primary amines is due to their inhibitory action on histaminase. The responses of the ileum to acetylcholine and 5-hydroxytryptamine were not potentiated.

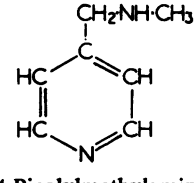
The experiments to be described were an outcome of observations on the action of 4-picolylamine upon the histaminase of human pregnancy serum. It was then noted that in sufficiently high concentrations picolylamine potentiated the response of the guinea-pig intestine to histamine. Since a similar potentiation of the response of the ileum by inhibitors of histaminase had been reported by Arunlakshana, Mongar & Schild (1954), it was thought to be interesting to find out if the action of picolylamine could be explained on a similar basis. This led to a study of related compounds, and these observations are described in this paper.

MATERIAL AND METHODS

Substances. The three isomers of picolylamine and the corresponding N-methyl derivatives, substituted in positions 2, 3 or 4, were obtained from Messrs Aldrich & Co.



4-Picolylamine



4-Picolylmethylamine

The structural formulae of 4-picolylamine and of 4-picolylmethylamine are shown above; the same side chains are present, in positions 2 or 3, in the two other amino and methylamino compounds.

Isolated guinea-pig ileum preparation. The piece of guinea-pig ileum was suspended in a bath, of a volume of 10 ml., in Tyrode solution. The temperature of the bath was 35° C, and a gas mixture of 95% oxygen + 5% carbon dioxide was bubbled through the bath. The

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magnification of the lever was 1:5. Histamine, acetylcholine and 5-hydroxytryptamine were present for 1 min; the substances to be tested as potentiators were added 30 sec earlier, and they remained in the bath through the period of contraction. Changes of bath fluid were automatic.

Manometric experiments. The preparation of histaminase ("diamine oxidase") used was an extract of an acetone-dried powder of pig kidney. The extract was prepared in 0.067 M sodium phosphate buffer of *p*H 7.4 as described by Blaschko & Hawkins (1950). In each manometer flask, the total reaction volume was 2.0 ml.; the main compartment of the conical manometer flask contained 1.6 ml. of the extract. The substrate, cadaverine or histamine as the dihydrochlorides, were added, with the picolylamines, from the side arm. The initial substrate concentration was 10^{-2} M. The potash tube contained 0.3 ml. N potassium hydroxide. The gas phase was oxygen, and the temperature of the manometer bath was 37.5° C. Percentage inhibition was calculated from the initial rate of the enzymic reaction, but the first 5-min period was not used for the calculations.

RESULTS

Observations on the guinea-pig ileum

4-Picolylamine. The effect of 4-picolylamine on the response of the ileum to histamine is shown in Fig. 1*a*. In this and all subsequent experiments the concentration of histamine (expressed as base) was 10^{-9}; the contraction elicited was well below the maximal response of the preparation to histamine. When 4-picolylamine was added in a concentration of 4×10^{-8}, the height of the contractions increased; this increase was seen as soon as the 4-picolylamine was added.

Contractions of the ileum of an order of magnitude similar to those in response to 10^{-9} histamine could also be produced by 5×10^{-11} acetylcholine chloride or by 4×10^{-8} 5-hydroxytryptamine. These contractions were not potentiated by 4-picolylamine; this is shown in Fig. 1*b* and *c*.

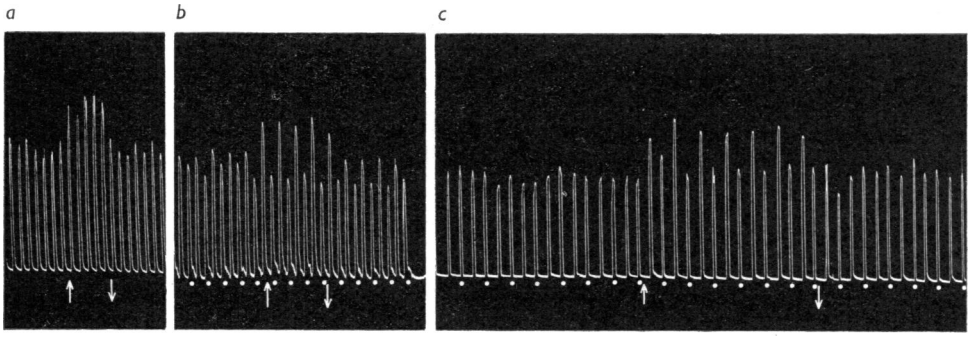

Fig. 1. Action of 4-picolylamine on the response of the isolated ileum of the guinea-pig to histamine. (*a*) Each contraction was in response to 10^{-9} histamine. Between arrows, 4×10^{-8} 4-picolylamine was present. (*b*) Alternate doses of 10^{-9} histamine (unmarked responses) and 4×10^{-8} 5-hydroxytryptamine (marked by a white dot). During the presence of 4×10^{-8} 4-picolylamine (between arrows) the response to histamine only was increased. (*c*) Alternate doses of 10^{-9} histamine (unmarked responses) and 5×10^{-11} acetylcholine chloride (marked by white dot). During the presence of 4×10^{-8} 4-picolylamine (between arrows) the response to histamine only was increased.

The effect of different concentrations of 4-picolylamine in the presence of a constant dose of histamine is seen in Fig. 2. The lowest concentration of 4-picolylamine at which a potentiation became evident was 10^{-10}; with increasing concentrations the effect increased, up to 10^{-5} 4-picolylamine. With 10^{-4} picolylamine no potentiation was seen. These results are similar to those obtained by Arunlakshana *et al.* (1954) for a number of other compounds. After washing out the high concentration of 4-picolylamine the preparation recovered fully. It may be mentioned that with preparations of 4-picolylamine exceeding 10^{-6} there was a slight rise in the base line, indicating that in high concentrations the amine by itself had a slight effect on the tone of the intestinal muscle.

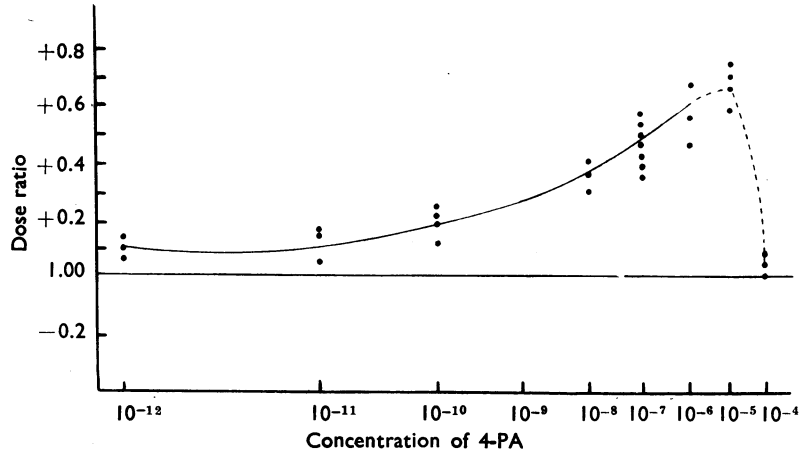

Fig. 2. Relationship between concentration of 4-picolylamine and potentiation of response to histamine. Abscissa: Concentration of 4-picolylamine in g/ml. logarithmic scale. Ordinate: Dose of histamine which, in the absence of 4-picolylamine, caused the same contraction as the standard dose in the presence of 4-picolylamine. With the standard dose, concentration of histamine base in the bath was $0{\cdot}5 \times 10^{-9}$.

When the dose of 4-picolylamine was kept constant at 10^{-7} and the doses of histamine were changed it was found that within the range of histamine concentrations tested the height of the contractions was increased by approximately the same amount (see Fig. 3).

2-Picolylamine and 3-picolylamine. The effect of these two isomers was similar to that of 4-picolylamine. The action of 2-picolylamine, in a concentration of 4×10^{-8}, is shown in Fig. 4*a*. The potentiation of the response to 10^{-9} histamine is clearly shown. Similar effects of 3-picolylamine are shown in Fig. 5 (periods 1 and 3). Thus, the potentiating action was seen with all three isomers of picolylamine, irrespective of whether the substitution was in positions 2, 3 or 4.

Usually it was noted that the contractions elicited by histamine immediately after the withdrawal of the picolylamines were a little smaller than they had been before the addition of the potentiator. This effect was only of short duration; it can be seen on Figs. 1, 4, 5 and 6. The phenomenon has not been fully analysed, but it was

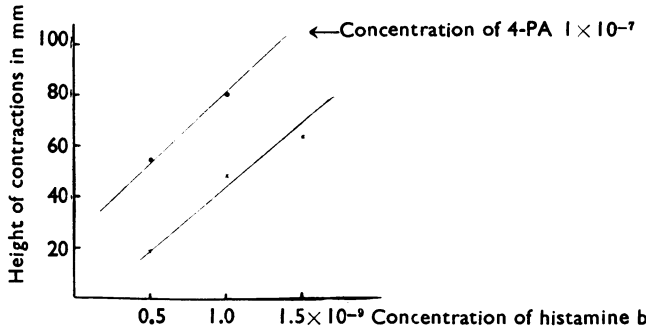

Fig. 3. Effect of a constant concentration of 4-picolylamine (10^{-7}) upon the response to varying doses of histamine. Abscissa: Histamine concentration in g/ml. Ordinate: Height of the contractions in mm.

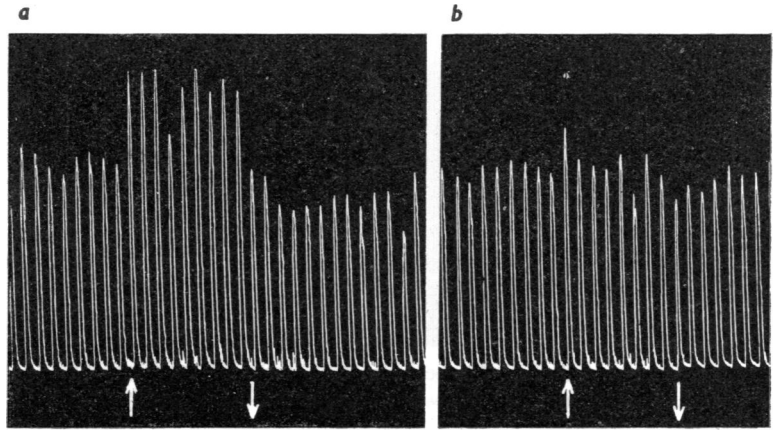

Fig. 4. The effects of 2-picolylamine and 2-picolylmethylamine on the response of the guinea-pig ileum to histamine. Histamine concentration: 10^{-9} g/ml. (*a*) Between arrows, 2-picolylamine (4×10^{-8}) was present. (*b*) Between arrows, 2-picolylmethylamine (4×10^{-8}) was present.

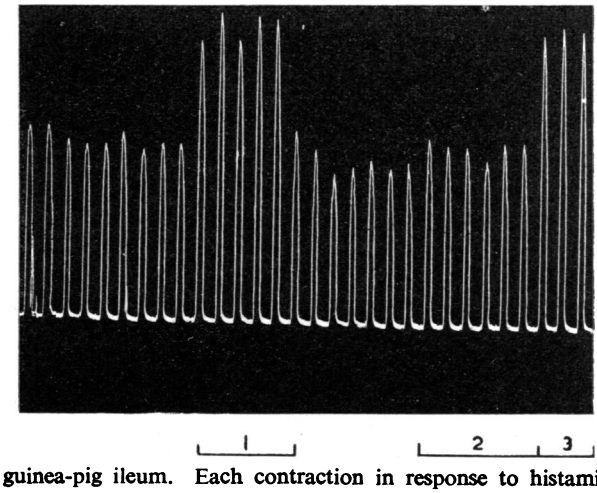

Fig. 5. Isolated guinea-pig ileum. Each contraction in response to histamine (10^{-9}). During periods 1 and 3, 3-picolylamine (4×10^{-8}) was also present; during period 2, 3-picolylmethylamine (4×10^{-8}).

found that a depression of the response to histamine occurred when 4-picolylamine was applied during a maximal potentiation brought about by aminoguanidine, one of the inhibitors of histaminase studied by Arunlakshana *et al.* (1954). It seems possible, therefore, that the picolylamines also have a very slight antihistaminic action, and that this may outlast the potentiating effect.

Picolylmethylamines. When the three isomers of picolylmethylamine, substituted in positions 2, 3 or 4, were tested in the same concentration as the primary amines, no potentiation of the response to histamine occurred. In the experiment of Fig. 6,

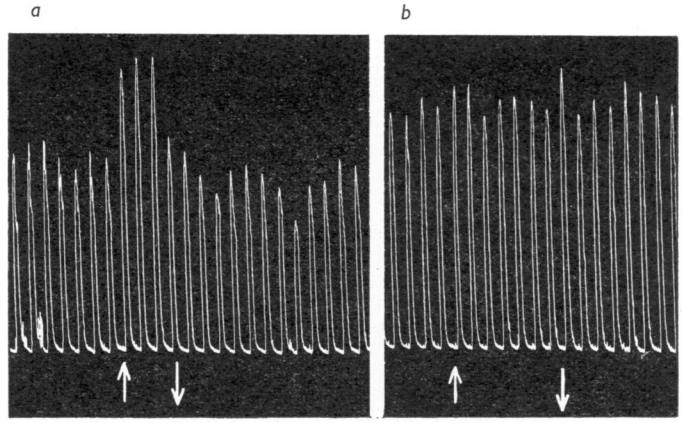

Fig. 6. Isolated guinea-pig ileum. The response to histamine (10^{-9} g/ml.) was potentiated by 4×10^{-8} g/ml. 4-picolylamine (*a*), but was not potentiated in the presence of 4×10^{-8} g/ml. 4-picolylmethylamine (*b*).

the effect of 4-picolylamine was compared with that of 4-picolylmethylamine. Results with 3-picolylmethylamine (Fig. 5, period 2) and with 2-picolylmethylamine (Fig. 4*b*) were similar: no potentiating effect was seen with any of the secondary amines.

Action of the picolylamine on the pig kidney oxidase

When the picolylamines were incubated with the pig kidney extract in a 10^{-2} M concentration, a very slight uptake of oxygen, probably just outside the limits of experimental error, was seen. Since it is known from earlier observations (Blaschko & Chruściel, 1959) that the three picolylamines are substrates of the pig serum oxidase, it must remain open whether this oxygen uptake is due to the action of the pig kidney oxidase or to a contamination of the preparation with pig serum.

All three isomers were inhibitors of the enzymic oxidation of cadaverine by the pig kidney enzyme. With 3- and with 4-picolylamine, the molar concentration of these amines that halved the uninhibited rate of the reaction was near 1.33×10^{-4} M; with 2-picolylamine it was near 4×10^{-4} M.

In contrast, the three picolylmethylamines, when tested in the same range of concentrations, were without inhibitory activity. Figs. 7*a* and 7*b* show the oxidation

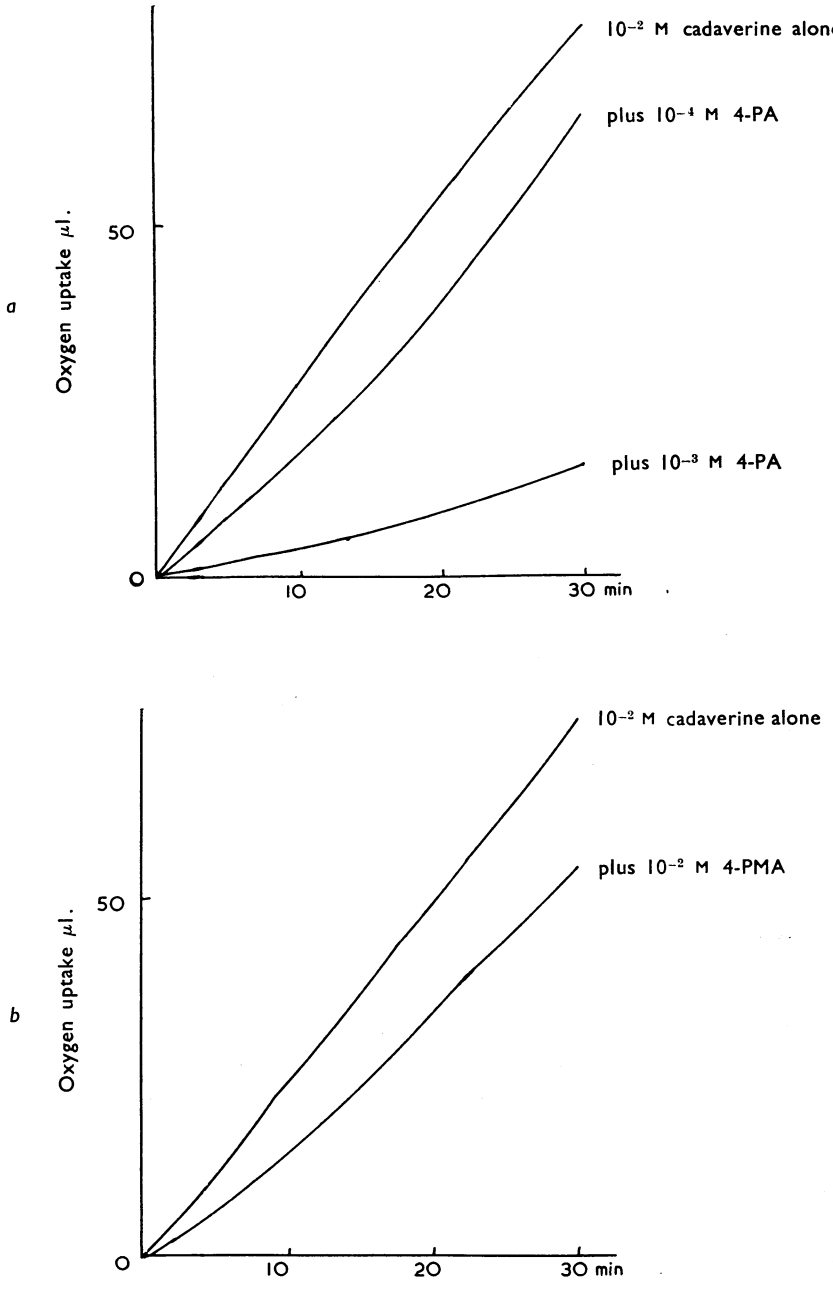

Fig. 7. The effects of 4-picolylamine (*a*) and 4-picolylmethylamine (*b*) upon the enzymic oxidation of cadaverine by an extract of acetone-dried powder of pig kidney. Abscissae: Time in min. Ordinates: μl. of O_2 consumed.

of cadaverine in the presence of 4-picolylamine and of 4-picolylmethylamine respectively. As can be seen, even in the highest concentration of 4-picolylamine that was tested, 10^{-2} M, the secondary amine reduced the rate of the enzymic reaction by less than 50%.

Histamine was a less convenient substrate for the manometric studies of the pig kidney enzyme than cadaverine, since it is much more slowly oxidized, but we have found an analogous difference between the effects of primary and secondary amines. In an experiment with 10^{-2} M histamine as substrate, 28 μl. of oxygen was consumed in the first 1.5 hr ; when 10^{-2} M 3-picolylamine was also present, the oxygen uptake was only 17 μl., but with 10^{-2} M 3-picolylmethylamine present it was 29 μl. In other words, inhibition of the histamine oxidation occurred with the primary, but not with the secondary, amine.

DISCUSSION

The response of the guinea-pig ileum to histamine was potentiated by all three isomers of picolylamine. There is good reason to believe that this effect is due to the inhibitory action of the picolylamines on the histaminase present in the tissue. This interpretation is strongly supported by the observation that the N-methylated derivatives did not potentiate the response to histamine. Histaminase belongs to a family of enzymes which sharply discriminates between primary amines and their N-methylated secondary analogues: only the former are oxidized. Although our knowledge of the chemistry of these enzymes is still very scanty, it is believed, on the basis of studies of enzyme inhibitors, that the prosthetic group, common to all members of this family, contains a carbonyl group. Such a group could be expected to react with primary, but not with secondary, amines. These enzymes, therefore, differ sharply from the intracellular amine oxidase (called " monoamine oxidase " by many authors), an enzyme that is not inhibited by carbonyl reagents and that does not discriminate between primary and secondary amines (see Blaschko, 1962).

It seems worth recalling that, in contrast to histaminase, the tissue receptors for histamine do not distinguish sharply between primary and secondary amines: the analogue of histamine with a methyl group replacing one of the hydrogens of the terminal amino group is pharmacologically fully active, like histamine (Vartiainen, 1935 ; Schild, 1947), but it is immune from attack by histaminase.

We have shown that the three isomers of picolylamine are inhibitors of the pig kidney enzyme. This preparation differs from the pig plasma oxidase, an enzyme that readily oxidizes the three amines (Blaschko & Chruściel, 1959). The secondary amines were without significant inhibitory action on the oxidation of cadaverine by the pig kidney oxidase. There is some doubt whether the enzyme that inactivates histamine is identical with diamine oxidase (see Kapeller-Adler & MacFarlane, 1962). However, if they are distinct, they must be closely related. We have satisfied ourselves that the enzymic oxidation of histamine by the pig kidney extract was also inhibited by the picolylamines ; the percentage inhibitions were less, as would be expected for a substrate with a higher affinity than cadaverine. It seems likely that in the tissues the conditions are more favourable for an inhibition of histaminase

than in the manometric experiment, where histamine is added in saturation concentration. In the intact tissue, the histamine concentration in the neighbourhood of the inactivating enzyme is likely to be much lower.

In conclusion, it can be said that the picolylamines, like the substances studied by Arunlakshana *et al.* (1954), exhibit pharmacological effects which they owe to their ability to inhibit an enzyme.

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