Brit. J. Pharmacol. (1951), 6, 68.

ANALYSIS OF CERTAIN INTERACTIONS OF NICOTINE WITH BRADYKININ AND HISTAMINE

BY

N. AMBACHE* AND M. ROCHA e SILVA

From the Department of Biochemistry and Pharmacodynamics, Instituto Biologico, São Paulo, Brazil

(Received October 16, 1950)

The method of using nicotine to produce paralysis of autonomic ganglia was first introduced by Langley in 1890. Since then this drug has been used from time to time as an analytical weapon in pharmacological studies on smooth muscle, in attempts to distinguish between substances which act directly on the muscle fibres and drugs which produce contraction by the stimulation of local ganglion cells. Unfortunately this analysis is often complicated by the following factors:

(*a*) The possibility of a direct antagonism between nicotine and some drugs but not others. This antagonism is observed when large doses of nicotine are kept in the bath in the presence of the stimulating agent. For instance, as we shall show, large doses of nicotine suppress the effect of histamine but not that of bradykinin. We believe that this antihistamine effect is a phenomenon of genuine antagonism; it is short-lived, and it usually lasts only as long as the nicotine is in the bath.

(*b*) The fact that nicotine is also an inhibitor of cholinesterase (Nachmansohn, 1939).

(*c*) The existence of an after-effect following large contractions evoked by smaller stimulant doses of nicotine, a phenomenon which we propose for the moment to call " residual inhibition." The nature of this inhibition is at present not quite clear. It bears some resemblance to a type of after-inhibition produced by other drugs, which was described by Cantoni and Eastman (1946) and was later re-studied by Beraldo and Rocha e Silva (1949). These authors observed a refractory state occurring after maximal contractions induced by acetylcholine and by pilocarpine, and lasting, in the guinea-pig's ileum, for as long as 10–15 min. afterwards. It was suggested by the authors quoted that the contraction produced an exhaustion of some essential energetic metabolite. It is likely, as suggested by Emmelin and Feldberg (1947), that the phenomenon which we have called " residual inhibition," and which was also observed by them after a stimulant dose of nicotine, may owe its origin to a similar process.

The antagonism of nicotine to histamine when the two are present together was observed earlier by Bernheim (1933), who came to the conclusion that the two drugs were " acting on the same receptor substance in the intestine." More recently Feldberg (1950) has stated that, apart from producing ganglionic block,

* On leave of absence from the Ophthalmological Research Unit, Medical Research Council, Institute of Ophthalmology, Judd Street, London, W.C.1.

large doses of nicotine " reduce the excitability of the muscle fibres as seen from the strongly reduced responses to histamine." In considering these two statements it seemed necessary to clarify the points (i) whether or not the antihistamine effect of nicotine was associated with a general reduction in excitability to all drugs, and (ii) whether it indicated a depression of the contractile mechanism. To put this matter to the test, we have availed ourselves of bradykinin, a newly described active principle, recently isolated by Rocha e Silva, Beraldo, and Rosenfeld (1949). This is a " slow " smooth-muscle-contracting substance, derived from plasma globulins by the proteolytic action of the venom of snakes of the genus *Bothrops* or of trypsin. We have found that, once the ganglion cells are paralysed by large doses of nicotine, the presence of the latter in the organ bath does not diminish the size of contractions produced by bradykinin; this result testifies to the absence of muscular depression by nicotine in concentrations of up to 3×10^{-4}. In contrast, as mentioned already, histamine contractions are suppressed by the same large amounts of nicotine. This distinction between histamine and bradykinin and the nature of the antihistamine and after-inhibitory actions of nicotine have been our main interests in the present experiments.

METHODS

The preparation of guinea-pig's ileum was made in the usual way; it was attached to a stainless steel-wire strut and suspended, with its lumen open at both ends, in a 7-c.c. organ bath filled with Tyrode's solution, and oxygenated with air. Contractions were produced at regular intervals (indicated in the Figures) by means of two " testing drugs," histamine or bradykinin, and were recorded with a compensated linear frontal-writing lever (Schild, 1947). Nicotine was not introduced into the bath until a " steady level " was attained in the response to the testing drugs. Drugs were added in a volume of 0.05–0.3 c.c. (rarely 0.5 c.c.) of distilled water. The preparation of bradykinin has been described before (Rocha e Silva, Beraldo, and Rosenfeld, 1949); different batches of " crude " bradykinin with an activity of about 1 " unit " per mg. (Prado, Beraldo, and Rocha e Silva, 1950 were used. The dilutions of nicotine were made from the free base.

RESULTS

Ganglion-cell paralysing concentrations of nicotine (3×10^{-4})

Our procedure was devised to maintain the ganglion cells in a state of paralysis throughout the experiment by the administration of nicotine at intervals. A standard dose of nicotine (2 mg.; referred to as the " paralysing dose ") was kept in the bath for at least one minute, only the first dose producing a short phase of stimulation followed by paralysis, and occasionally by a contraction just after the removal of nicotine by washing (asterisks in Figs. 4 and 5). Time was allowed for the subsidence of this " wash-out contraction " before retesting the gut with a second or third dose of 2 mg. nicotine, neither of which elicited contraction. On the contrary, these repeat doses appeared to hasten relaxation after the " wash-out contraction." Subsequently, the 2-mg. dose of nicotine was repeated from time to time, without producing contractions either on introduction or after the wash-out, as shown in Figs. 2 and 3.

Bradykinin.—When the intestine was treated with these paralysing doses of nicotine, and bradykinin was added subsequently, either before or after removal of

nicotine from the bath, there was no significant decrease in the response to bradykinin. This is shown quite clearly in Figs. 1 and 2. Only once in ten trials (three experiments) was the response to bradykinin depressed in the presence of the paralysing dose of nicotine. In thirteen other trials (also in three different experiments), in

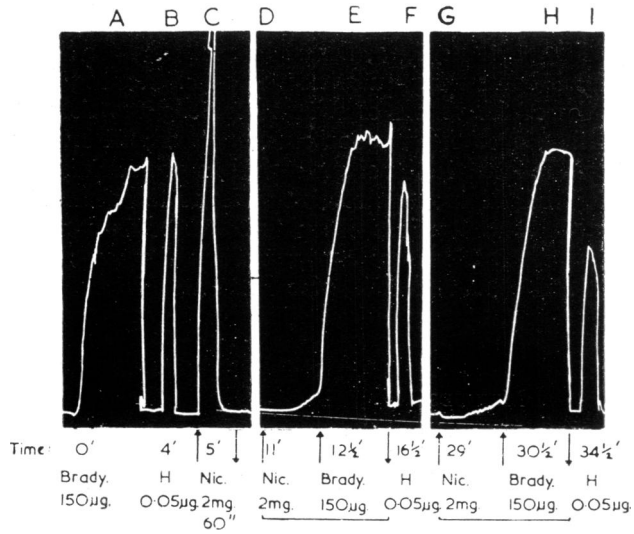

FIG. 1.—Guinea-pig ileum in 7-c.c. bath. ↑ indicates addition of drug, ↓ indicates that the solution in the bath was changed. The first dose of nicotine, at C, produced a powerful contraction followed rapidly by relaxation. Subsequent doses at 6½ and 18 min. (not shown; both left in the bath for 2 min.) and at D and G had no such effect, indicating the persistence of ganglion-cell paralysis throughout the experiment. The effect of 150 µg. of bradykinin (at E and H) in the presence of nicotine was no smaller than its initial effect at A in the absence of nicotine. The experiment shows that a large dose of nicotine *per se* does not depress the contractile mechanism of intestinal muscle.

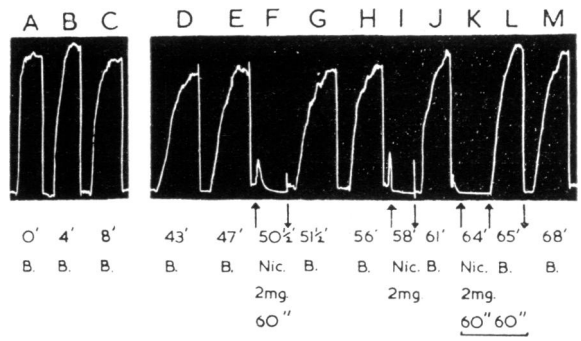

FIG. 2.—Preparation as in Fig. 1. Muscle tested with a standard dose of 50 µg. bradykinin for 60 sec. throughout. A, B, C, initial response to bradykinin. Between C and D the ganglion cells were paralysed with 2 mg. nicotine (at 11 min.; not shown). This paralysis was maintained by frequent repetition of the same dose of nicotine at intervals for the remaining 1¼ hours of the experiment. During this time there did not appear to be any significant difference between (*a*) the response to bradykinin in the presence of nicotine (at L); (*b*) the response to bradykinin after, or just after, a dose of nicotine had been washed out (at G, J, and M); and (*c*) the response to bradykinin alone.

which the bradykinin was added *after* washing out the paralysing dose of nicotine, only twice was there any detectable after-inhibitory effect upon bradykinin, while eleven times no trace of inhibition could be detected. These experiments point to the conclusion that the contractile mechanism in the plain muscle of the guinea-pig intestine is not affected by these doses of nicotine.

Histamine.—In contrast with the resistance of the bradykinin-response to nicotine, the action of histamine was definitely affected by the paralysing doses of nicotine when the two were present together in the organ bath. Without exception, 2 mg. nicotine produced a strong reduction in the response to a test dose of histamine, or even its suppression (Fig. 3). This antagonism was observed in nine out of nine

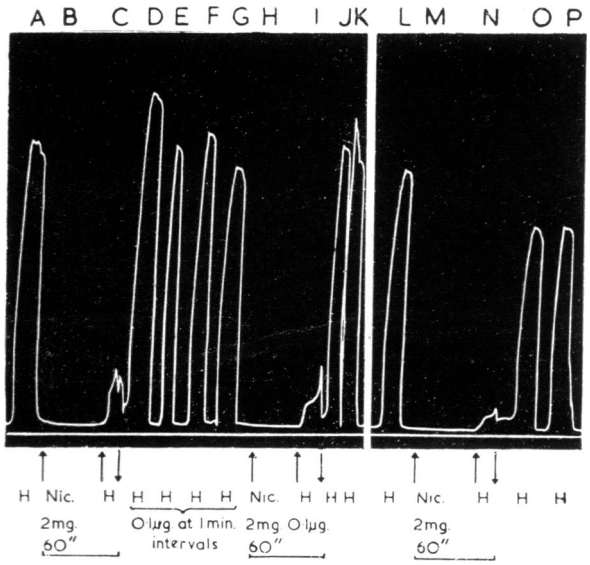

FIG. 3.—Preparation as in Fig. 1. The record illustrates the direct antagonism of a large dose (2 mg.) of nicotine to histamine. The ganglion cells were paralysed with 2 mg. nicotine some time before A, and were kept paralysed throughout the experiment by repeating the nicotine at frequent intervals. The effect of a standard dose of histamine (0.1 μg.) was antagonized by the presence of nicotine in the bath, at C, I, and N. After removal of the nicotine the histamine effect recovered at D, and again at J and O, within 1 minute of the nicotine being washed out. Bradykinin was not antagonized by the same dose of nicotine earlier in this experiment.

trials (two experiments). It may be tentatively considered as a "competitive" type of phenomenon, because the antihistamine effect disappeared quite soon after the nicotine had been washed out (Fig. 3, D, J, and O), although the ganglion cells remained in a paralysed state for a considerable time thereafter, as shown by the ineffectiveness of further doses of nicotine repeated at intervals. Thus, in contrast with what happened after stimulating doses of nicotine (see next section), there was little or no residual inhibition after paralysing doses. After the removal of nicotine, the muscle may regain its full sensitivity to histamine within 60 sec. (Fig. 3, J), but a slight depression of the histamine response after the withdrawal of nicotine was observed on one or two occasions (for instance, Fig. 1 at I).

Stimulating doses of nicotine (100–150 μg.)

A small dose of nicotine (10 μg.), although capable of evoking a contraction, was not followed by a decrease of subsequent responses to histamine. A larger stimulant dose (100 to 150 μg.) produced both effects—first the contraction, and subsequently, after the removal of nicotine, a variable reduction in the response to successive test-doses of histamine. This phase of post-nicotine "residual inhibition" is of several minutes' duration. The time-course of recovery and the shape of the inhibitory curve after nicotine varied slightly. For instance, in Fig. 4 the second histamine-contraction (at F) after the first dose of nicotine was considerably more depressed than the preceding one at E (cf. Beraldo and Rocha e Silva, 1949; Fig. 4A). But, after the next 150-μg. dose of nicotine at N, the first histamine-contraction, at O, was more depressed than the second at P. Allowing for this variability, the curve described by this inhibitory effect resembled, in many instances, that of the Cantoni and Eastman (1946) phenomenon alluded to in the introduction. This type of "residual inhibition" after 100–150-μg. doses of nicotine was observed regularly in a total of sixteen out of sixteen trials, in six experiments on different preparations.

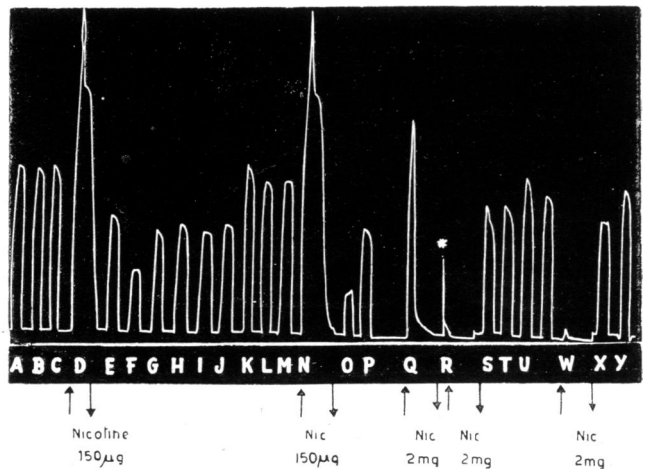

FIG. 4.—Preparation as in Fig. 1. With the exception of the responses to nicotine, which was left in the bath for 60 sec., all contractions were produced by a standard dose of histamine (0.1 μg.) every 1–2 min. The experiment illustrates: (1) the persistence of residual inhibition for 11 min. (E–K) after the first stimulant dose (150 μg.) of nicotine; (2) further inhibition after another 150 μg. of nicotine at N. Two paralytic doses (2 mg.) of nicotine, at Q and R, were followed by smaller inhibitions (S, T, and U), as was a third dose (at W), although the dose of nicotine was more than 10 times greater than at D or N. The contraction marked by an asterisk occurred after the wash-out following Q, and before R; time was allowed for its subsidence.

The " residual inhibition " produced by successive stimulant doses of nicotine is additive; thus, in Fig. 5 the second 100-μg. dose of nicotine at F was so spaced as to fall in the recovery period after the first at B (and J in that of F); each dose was followed by an increase in the depth of inhibition produced by its predecessor.

" Residual inhibition " can be distinguished from the so-called competitive type of antagonism between large doses of nicotine and histamine described above. The two processes appear to be quite different for the following reasons: (i) whereas the " antagonism " lasts only as long as nicotine is in the bath, the " residual "

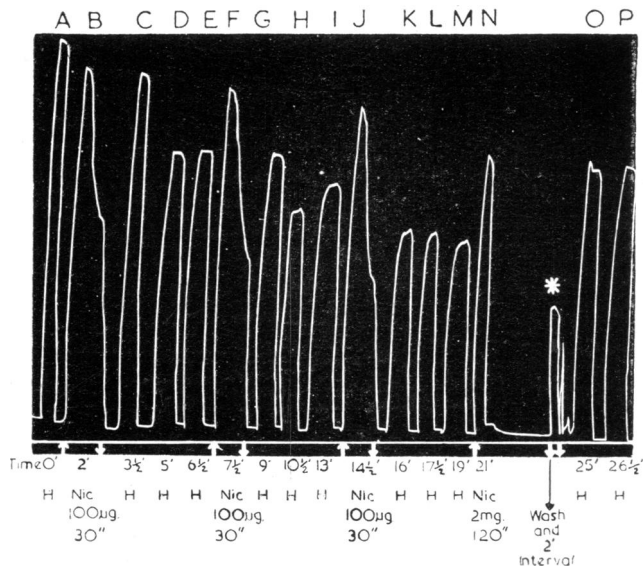

FIG. 5.—Preparation as in Fig. 1. Muscle tested with a standard dose (0.15 μg.) of histamine at regular intervals every 1½ min. Summation of residual inhibition produced by successive stimulant doses of 100 μg. nicotine for 30 sec. at B, F, and J. A paralytic dose of 2 mg. nicotine for 120 sec. at N appears to produce less residual inhibition (at O and P). The contraction marked with an asterisk occurred after the nicotine had been washed out. An interval of 2 min. was allowed for its subsidence.

effect is of long duration, lasting several minutes after the removal of nicotine, and may take some time before it reaches its maximum ; and (ii) the residual inhibition could not be observed after previous paralysis of the ganglion cells; and, as shown in Figs. 4 and 5, when the effect of small and large doses of nicotine was examined successively, the large dose appeared to produce less residual inhibition than the small dose (e.g., Fig. 4, X).

Discussion

On the basis of the experiments presented in this paper the following conclusions can be reached. Firstly, nicotine in concentrations of up to 3×10^{-4} (*a*) has no depressing effect upon the contractile mechanism in the smooth muscle of the guinea-pig's ileum, and (*b*) does not affect its excitability and responsiveness to all drugs. This point is clearly demonstrated by the fact that large doses of nicotine did not reduce the size of the contractions evoked by bradykinin, even when the two drugs were present together.

Secondly, interaction between nicotine and histamine is of two kinds. There is, first, the antagonism of the large dose of nicotine to histamine, when the two are present together. Since we have excluded (with bradykinin) the possibility of a harmful effect of nicotine upon the contraction process proper, this second effect of nicotine upon histamine-contractions must receive some other explanation. Since this antagonism disappears rapidly almost as soon as the nicotine is washed out, it appears to originate in a phenomenon of a " competitive " nature.

The second type of interaction between nicotine and histamine is the so-called " residual inhibition " of histamine, which is seen for several minutes after the smaller stimulating dose (100–150 μg.) of nicotine. This may be an after-effect of the contraction itself (or of the release of acetylcholine in the gut which led to the contraction), rather than a primary effect of the nicotine, since the larger " paralysing " dose of nicotine produced less residual inhibition. The mechanism of this " residual inhibition " raises a further point of theoretical interest, namely whether the ganglion cells play any part in this and allied phenomena. If the explanation proposed by Cantoni and Eastman (1946) for the phenomenon which they observed be accepted, then a temporary exhaustion of an essential energetic metabolite after the contraction produced by the stimulating dose of nicotine might also be assumed; this " residual inhibition " would then be comparable to that produced by any maximal contraction set off by such distinct substances as acetylcholine, histamine, and pilocarpine. Paralysis of ganglion cells appears to play no role in the production of this long-lasting residual inhibition after nicotine, since the larger paralysing dose of nicotine appeared to produce less residual inhibition than the smaller stimulating doses. That the Cantoni-Eastman effect is not itself due to ganglionic paralysis was further shown by the fact that it could still be obtained as a sequel to maximal contractions induced by strong doses of acetylcholine (10 and 20 μg. in two different experiments), in preparations of which the ganglion cells were previously paralysed with nicotine. One of these experiments is illustrated in Fig. 6. Although the ganglion-cell paralysis was maintained between A and F (as tested again with 2 mg. of nicotine at F, which maintained paralysis thereafter), the refractoriness towards histamine, which is characteristic of the Cantoni-Eastman effect, developed as usual after the 20-μg. dose of acetylcholine.

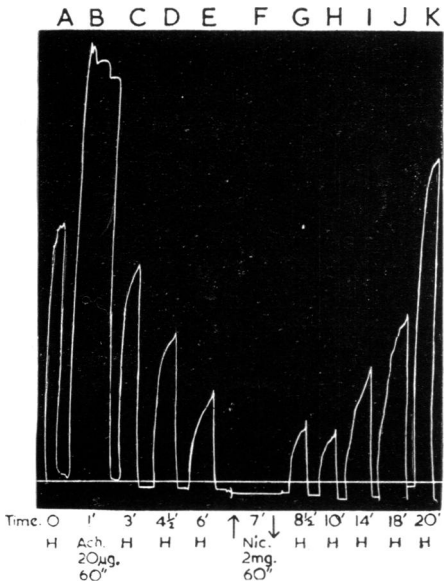

FIG. 6.—Continuation of Fig. 5 after an interval of 5½ min. Refractory state after a large contraction produced by acetylcholine (Cantoni and Eastman effect). Muscle tested at intervals with a standard dose of histamine (0.15 µg.). Ganglion-cell paralysis was maintained by a 2-mg. dose of nicotine (not shown) administered 3 min. before A, and left in the bath for 2 min.; when repeated at F, this dose (2 mg. for 60 sec.) was ineffective, and the ganglion cells may be presumed to have been paralysed throughout the experiment, yet the refractory state which follows a large dose of acetylcholine (20 µg. for 60 sec. at B) developed as usual, and appears to be independent of ganglion-cell function.

SUMMARY

1. The smooth muscle in the guinea-pig's ileum is not depressed by concentrations of nicotine which are used to produce ganglion-cell paralysis (3×10^{-4}).

2. This was shown in experiments in which the intestine, maintained in a prolonged state of ganglion-cell paralysis by frequent repetition of 2-mg. doses of nicotine, showed no decrease in its response to bradykinin, either in the presence of nicotine or after its removal.

3. In contrast, once the ganglion cells are paralysed, the action of histamine is antagonized by the presence of a 2-mg. dose of nicotine in the bath, but shows recovery almost as soon as the nicotine has been washed out.

4. Stimulating doses of nicotine (100–150 µg. for 30–60 sec.), producing large contractions, leave in their wake a state of " residual inhibition " which may last up to 12 min. (Fig. 4). This appears to be an after-effect of the contraction, and resembles an effect described by Cantoni and Eastman (1946) after other contracting drugs.

The authors are indebted to Mr. Jayme Ferraz for technical assistance. This work was done during the tenure by one of them (N.A.) of a Brazilian Government Fellowship.

REFERENCES

Beraldo, W. T., and Rocha e Silva, M. (1949). *J. Pharmacol.*, **97**, 388.
Bernheim, F. (1933). *J. Pharmacol.*, **48**, 67.
Cantoni, G. L., and Eastman, G. (1946). *J. Pharmacol.*, **87**, 392.
Emmelin, N., and Feldberg, W. (1947). *J. Physiol.*, **106**, 48.
Feldberg, W. (1950). *XVIII International Physiological Congress Abstracts*, p. 197.
Langley, N. (1890). *J. Physiol.*, **11**, 123.
Nachmansohn, D. (1939). *C.R. Soc. Biol., Paris*, **130**, 1065.
Prado, J. C., Beraldo, W. T., and Rocha e Silva, M. (1950). *Arch. Biochem.*, in the press.
Rocha e Silva, M., Beraldo, W. T., and Rosenfeld, G. (1949). *Amer. J. Physiol.*, **156**, 261.
Schild, H. O. (1947). *Brit. J. Pharmacol.*, **2**, 189.