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DUAL ACTION OF NITROPARAFFINS ON THE GUINEA-PIG ILEUM

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Nitroparaffins act on the guinea-pig ileum by several mechanisms. They produce contraction partly by ganglionic stimulation and partly by direct liberation of transmitter from the nerve endings. They also inhibit the response to acetylcholine and nicotine, but interfere less with the action of histamine, serotonin or bradykinin. The two opposite effects of the nitro compounds are independent of each other. Within the homologous series of normal nitroparaffins, the excitatory action diminishes and the inhibitory effect increases with the lengthening of the carbon chain.

The mechanism of drug effects on smooth muscle organs represents a difficult problem due to the complexity of the reacting system involved and the many sites available for interaction. Thus, excitation or inhibition may take place not only at any part of the nervous system present in the wall of such organs, especially at the ganglion cells, but also at the neuromuscular junction or on the smooth muscle cell itself. In many instances a drug may attack at several sites, but the dosage required for the different actions is not identical. For example, morphine depresses the response to drugs which stimulate nervous structures in the intestinal wall causing release of acetylcholine (Schaumann, 1955, 1956), but higher doses of the alkaloid also counteract direct stimulation of the smooth muscle fibres (Lewis, 1960). Morphine is thus a representative of agents which produce the same type of effect—excitation or inhibition—at more than a single site. In such cases, it is a laborious task to separate the components of the overall effect.

Almost all agents, active on smooth muscle organs, are either bases or acids. In the present study we have selected a third type, embodying a strongly polar but uncharged group at the end of a non-polar chain, viz., the nitroparaffins. It is well known that these compounds range among the aliphatic substances with the highest dipole moments (Groves & Sugden, 1935). It was found that the members of this series may act on the intestine in two or more different ways, the main action shifting within the homologous series from excitation to inhibition. As will be shown in this study, the combination of effects exerted by a given nitroparaffin can be resolved into excitatory and inhibitory components. Experiments with nitrobenzene are also included, since its actions are qualitatively similar to those of the aliphatic nitro compounds.

METHODS

Guinea-pigs were killed by a blow on the neck and the small intestine used immediately, or after 24 hr storage in a refrigerator at +5° C. Loops of about 3 cm length were suspended at 37° C in a 50 ml. organ bath containing Tyrode solution. The gut contractions were recorded by means of an isotonic lever, using 5- to 10-fold magnifications.

For comparison of inhibitory effects the I 50 values, measuring 50% reduction of the contraction provoked by 2 to 6 ng/ml. of acetylcholine, were first determined on separate loops. The I 50 concentrations of the members of the nitroparaffin series were then retested on a single piece of gut. For reasons explained under Results, after each application of a nitro compound, the ileum was incubated for 5 min with 20 ng/ml. of acetylcholine. After each group of three nitroparaffins, nitromethane was applied to check whether the preparation had preserved its sensitivity.

The lower nitroparaffins used were products of the Commercial Solvents Corporation. Nitro-n-hexane was obtained from Dr H. Feuer, and the n-pentane, n-heptane and n-octane derivatives as well as the nitrocycloalkanes from Professor N. Kornblum, both of Purdue University, Lafayette, Indiana. All nitroparaffins were redistilled before use. The standard concentrations in distilled water, used in the present experiments, are included in Table 1.

Bradykinin was the synthetic peptide of the Sandoz Pharmaceutical Corporation, Basle, Switzerland.

Rat mast cells were collected from the peritoneum after two intraperitoneal injections of saline, as described previously (Bergmann, Leon, Preiss & Chaimovitz, 1962). They were spun down, washed once with saline and used immediately for incubation with nitroparaffins.

RESULTS

Stimulation and inhibition of guinea-pig ileum under the influence of nitroparaffins. Fig. 1 shows the succession of effects obtained with nitroethane, viz., a contraction, followed by relaxation and partial inhibition of the response to acetylcholine. This characteristic sequence is qualitatively the same for all members of the nitroparaffin series, but the quantitative relation between the various components of the response in Fig. 1 fluctuates from one nitro compound to the other.

The inhibitory power of the nitroparaffins can be expressed as the concentration (I 50) required to reduce by 50% a contraction provoked by acetylcholine after a standard period of 3 min incubation (as in Fig. 1). On this basis, the antispasmodic effect is increased by extending the chain length of the nitroparaffins (Table 1). In parallel with the enhanced inhibitory power, it also becomes more difficult to restore the sensitivity of the intestinal loop to acetylcholine by washing.

It is more difficult to evolve a satisfactory method for comparison of the stimulatory effects of the nitroparaffins. First of all, repeated applications of the same or different nitro compounds are feasible only under specific conditions, as discussed below. Secondly, for the lower members of the series, the dose-response curve shows a maximum (Fig. 2), but the same function cannot be determined for higher nitroparaffins because of their limited water solubility. Finally, comparison may be based on the molar concentrations, giving equal height of contraction. This again is of doubtful value in the present series, since the increasing inhibitory power of the higher nitro derivatives may prevent full manifestation of their excitatory activity. For these reasons, the amplitude of contraction, evoked by the I 50 concentrations given in Table 1, was chosen arbitrarily for comparison. There-

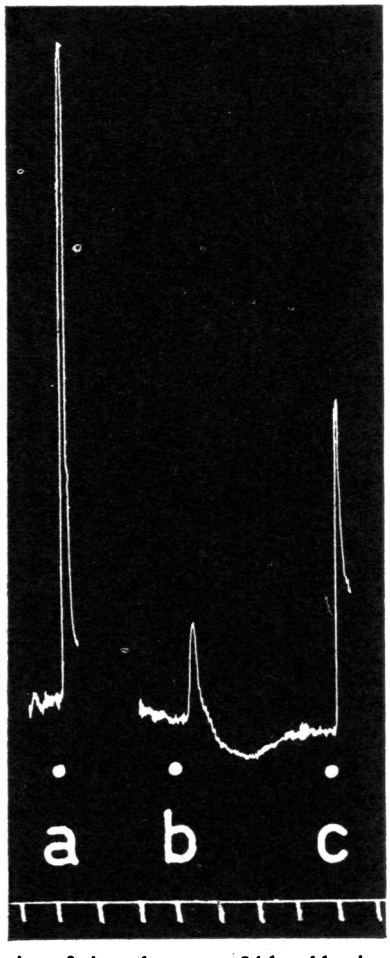

Fig. 1. The three stages of action of nitroethane on a 24-hr-old guinea-pig ileum. *a*, Acetylcholine, 3 ng/ml.; between *a* and *b*, 3 washings. *b*, Nitroethane, 1 : 3,000, produces first contraction, then relaxation. *c*, 4 min later, without washing, acetylcholine, 3 ng/ml., was added. Note 50% inhibition at *c*. Time in min.

fore, only in a very limited sense can it be stated that the excitatory effect decreases with increasing chain length. This is also discernible in Fig. 2. The maximal contraction which can be elicited by nitroethane is much below the corresponding peak of the curve characteristic for nitromethane. Beyond the n-hexane derivative, it is practically impossible to induce contraction.

Interaction of nitroparaffins with antagonists to smooth muscle stimulants. In order to elucidate the mechanism of excitation of the smooth muscle by aliphatic nitro compounds, a number of potential antagonists were studied. Atropine, in a concentration effective against acetylcholine, does not inhibit contractions induced by *nitromethane* (Fig. 3), the latter thus resembling nicotine. Only 20 to 30 times higher doses of atropine suppress the action of nicotine or of the nitroparaffin. A further manifestation of the pharmacological similarity of these two agents is found

TABLE I

INHIBITORY AND EXCITATORY ACTIVITY OF NITROPARAFFINS

I 50 values denote the dilutions reducing by 50% a contraction evoked by acetylcholine. The values in this column were determined on a single intestinal loop against 4 ng/ml. of acetylcholine. *Dissolves only with difficulty upon warming and shaking

Compound	Standard solution in water (v/v)	I 50 (v/v)	Relative excitatory activity of I 50 concentrations
A. *Straight-chain nitroparaffins*			
Nitromethane	1 : 20	1 : 650	100
Nitroethane	1 : 70	1 : 2,300	60
1-Nitropropane	1 : 170	1 : 5,700	40
1-Nitro-n-butane	1 : 200	1 : 7,700	40
1-Nitro-n-pentane	1 : 600	1 : 16,000	25
1-Nitro-n-hexane	1 : 1,000	1 : 33,000	15
1-Nitro-n-heptane	1 : 5,000*	1 : 70,000	5
1-Nitro-n-octane	1 : 6,000*	1 : 145,000	0
B. *Branched-chain nitroparaffins*			
2-Nitropropane	1 : 80	1 : 2,700	43
2-Nitro-2-methyl-propane (" tert. nitrobutane ")	1 : 150	1 : 2,100	87
C. *Cyclic nitro derivatives*			
Nitrocyclopentane	1 : 400	1 : 25,000	20
Nitrocyclohexane	1 : 600	1 : 15,000	15
Nitrobenzene	1 : 1,000	1 : 35,000	35

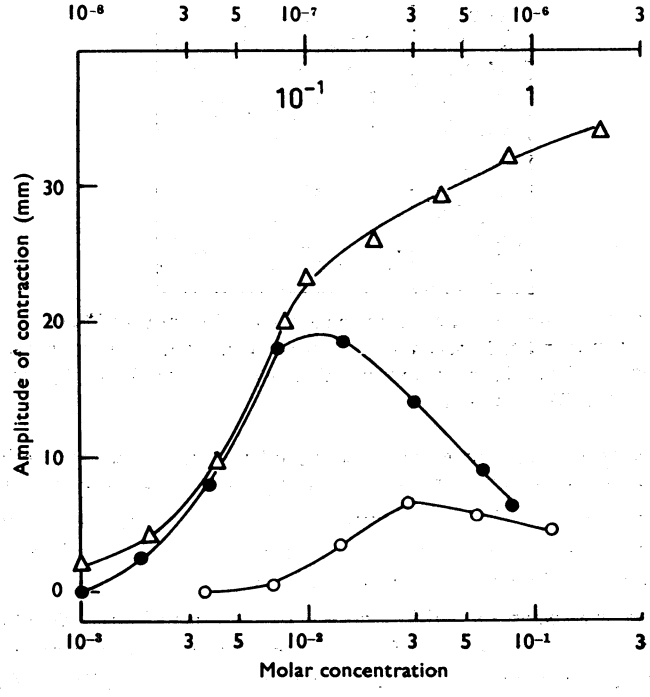

Fig. 2. Amplitude of contraction of fresh guinea-pig ileum as function of logarithm of dose. △ — △, Acetylcholine (upper abscissa, upstroke); ● — ●, nitromethane (lower abscissa); ○ — ○, nitroethane (upper abscissa, downstroke). Between two points of the nitroparaffin curves, the intestinal loop was incubated with 20 ng/ml. acetylcholine for 5 min.

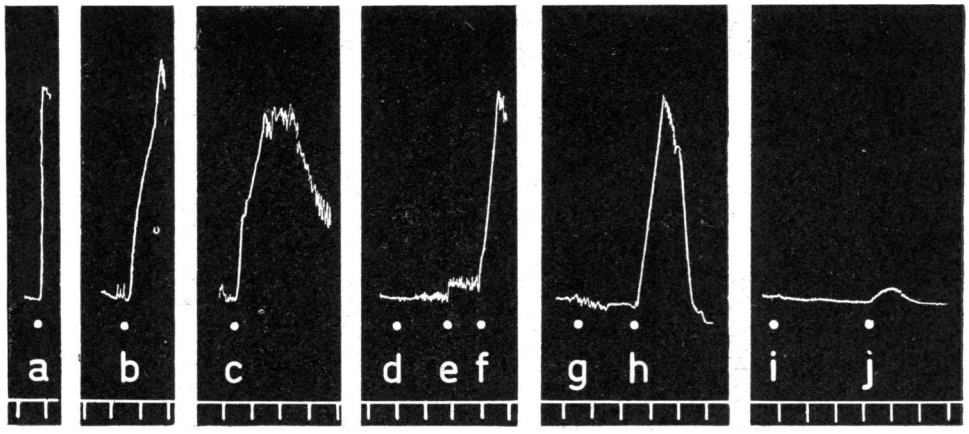

Fig. 3. Effect of low and high concentrations of atropine on nicotine- or nitromethane-induced contractions of the ileum. Between sections, the gut was washed 3 times. *a*, Acetylcholine, 6 ng/ml.; *b*, nicotine, 0·6 μg/ml.; *c*, nitromethane, 1 : 500; *d*, atropine, 10 ng/ml.; *e*, 2 min later, without washing, acetylcholine, 6 ng/ml.; *f*, 90 sec later, without washing, nicotine, 0·6 μg/ml.; *g*, atropine, 200 ng/ml.; *h*, 2 min later, without washing, nitromethane, 1 : 500; *i*, atropine, 200 ng/ml.; *j*, nitromethane, 1 : 500.

in the shape of the curves, reproducing the course of the intestinal contraction. Both agents provoke large peristaltic waves at the height of the response and are unable to maintain the maximal tonus of the smooth muscle over a prolonged period (Figs. 4 and 6).

These results suggest that the nitroparaffins act by releasing acetylcholine as it is well known that atropine counteracts exogenous acetylcholine much more effectively than the intrinsic transmitter (Schaumann, 1955, 1956).

It was therefore indicated to study the influence of morphine. The alkaloid, at concentrations of 60 ng/ml., had no measurable effect on contractions provoked by acetylcholine, but abolished completely stimulation of the gut by nitromethane (Fig. 4). On the other hand, the *inhibitory* action of the nitro compound itself against responses to acetylcholine remained unchanged. Likewise, relaxation of the gut by 1-nitropropane, for example, was not abolished by morphine (Fig. 5). This result clearly shows that the two phases in the sequence, characteristic for the overall effect of nitroparaffins on the ileum (Fig. 1), are independent of each other.

Whereas the foregoing tests pointed to the similarity of the stimulatory action of nicotine and the nitroparaffins, a disparity came to light in experiments with hexamethonium. A dose of 5 μg/ml., which suppressed completely the response to nicotine, reduced the contraction of the ileum to nitromethane only to about one-half (Fig. 6). Even 5 times higher concentrations of the methonium compound could not produce a further depression.

Mechanism of nitroparaffin-induced contractions of the ileum. Evidently, in the first phase of nitroparaffin action, leading to smooth muscle contraction, two

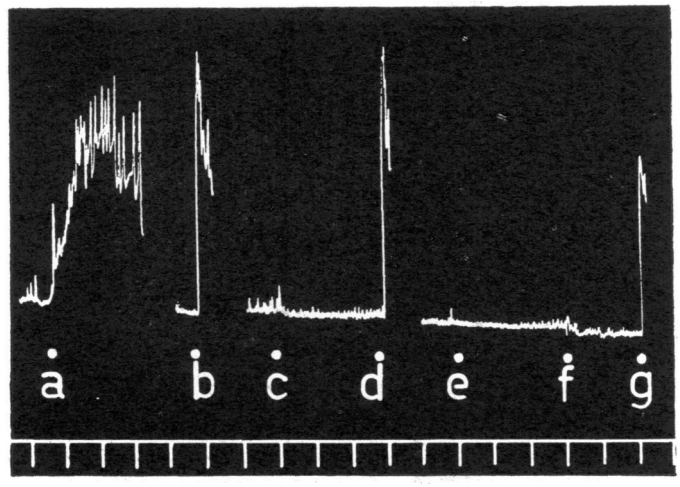

Fig. 4. Influence of morphine on nitromethane-induced contraction of fresh guinea-pig ileum. *a*, Nitromethane, 1 : 650. Note large peristaltic waves during rising and at stationary phase of contraction. Between *a* and *b*, 3 washings. *b*, Acetylcholine, 3 ng/ml.; *c*, morphine, 60 ng/ml.; *d*, 3 min later, without washing, acetylcholine, 3 ng/ml. ; between *d* and *e*, 3 washings; *e*, morphine, 60 ng/ml. ; *f*, 3 min later, without washing, nitromethane, 1 : 650 ; *g*, 2 min later, acetylcholine, 3 ng/ml. Note 33% reduction of height of contraction as compared to *b* and *d*.

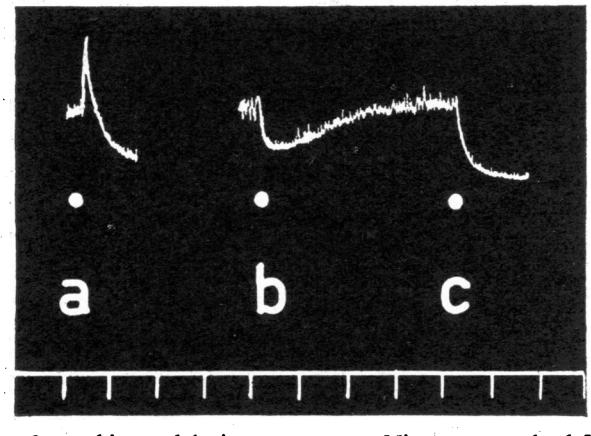

Fig. 5. Interaction of morphine and 1-nitropropane. *a*, Nitropropane, 1 : 1,500. Note biphasic action. *b*, Morphine, 60 ng/ml. ; *c*, 3 min later, without washing, nitropropane, 1 : 1,500. Note that the excitatory phase has been completely abolished, while relaxation persists.

effects are discernible: excitation of ganglionic cells in the intestinal wall and stimulation of the nerve terminals at the neuromuscular junctions. This hypothesis gains further support from the following observations: (1) Ageing of the ileum by storage in a refrigerator diminishes the excitatory effect of the nitroparaffins, without impairing measurably their inhibitory action. (2) Repeated applications of a short-chain nitroparaffin produce responses of diminishing amplitude (Fig. 7). This

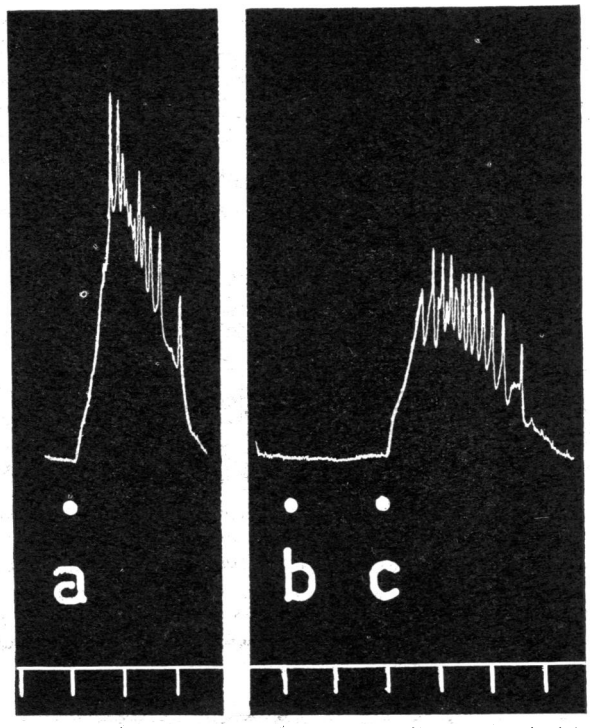

Fig. 6. Effect of hexamethonium on contractions of fresh ileum provoked by nitromethane. *a*, Nitromethane, 1 : 650; after 3 washings, at *b*, hexamethonium, 5 μg/ml.; no visible effect; *c*, 2 min later, without washing, nitromethane, 1 : 650. Note 40% depression of amplitude of contraction as compared to *a*.

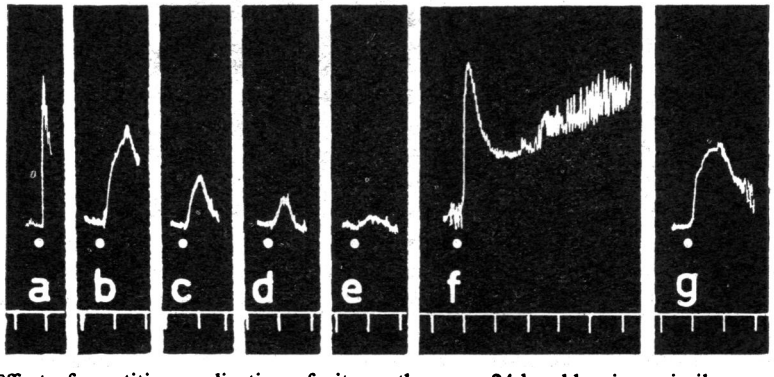

Fig. 7. Effect of repetitive application of nitromethane on 24-hr-old guinea-pig ileum. Between sections, 3 washings each. *a*, Acetylcholine 4 ng/ml.; *b* to *e*, nitromethane, 1 : 500. Note rapidly declining amplitude of contraction. *f*, 5 min incubation with acetylcholine, 4 ng/ml. Note that acetylcholine effect is identical with that in *a*. *g*, Nitromethane, 1 : 500. Note almost complete (80%) recovery.

decline can be prevented by intercalating incubation with acetylcholine. In accordance with these results is the finding that after a second portion of nitroethane, added to the bath without washing out the previous dose, contraction is even more reduced (Fig. 8). These observations made it necessary to incubate the intestinal loop with acetylcholine for at least 5 min each time a new test with a nitroparaffin was to be performed. (3) A dosage of nitromethane, which is too small to stimulate the gut, enhances the acetylcholine effect (Fig. 9). Sometimes, especially with nitroparaffins of considerable inhibitory power such as nitrocyclopentane, the augmentation became apparent only after the first washing, as in Fig. 10, but was completely eliminated by further changes of the bath fluid. It is thus unlikely that the enhancement of the response to acetylcholine was due to release by the nitro compound of subliminal concentrations of intrinsic mediator. On the other hand, it is conceivable that the nitroparaffin changes the permeability of the nerve membrane sufficiently to pave the way for subsequent release of transmitter by acetylcholine.

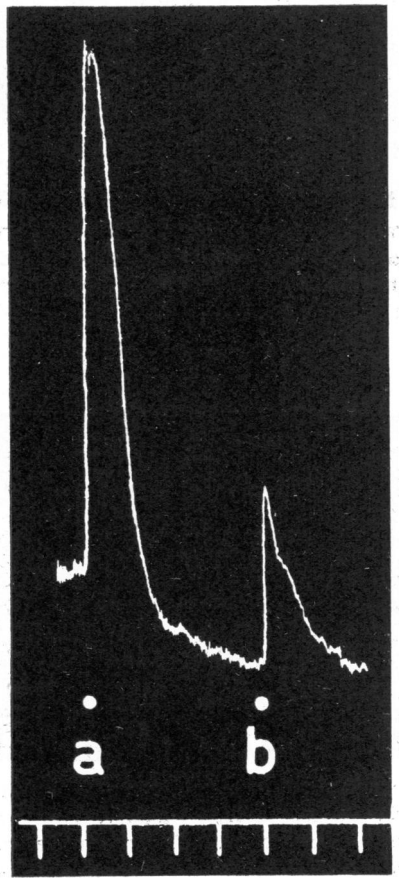

Fig. 8. Lessened effect of second dose of nitroethane on fresh ileum. Both at *a* and *b*, with an interval of 4 min, nitroethane, 1 : 2,000, was added.

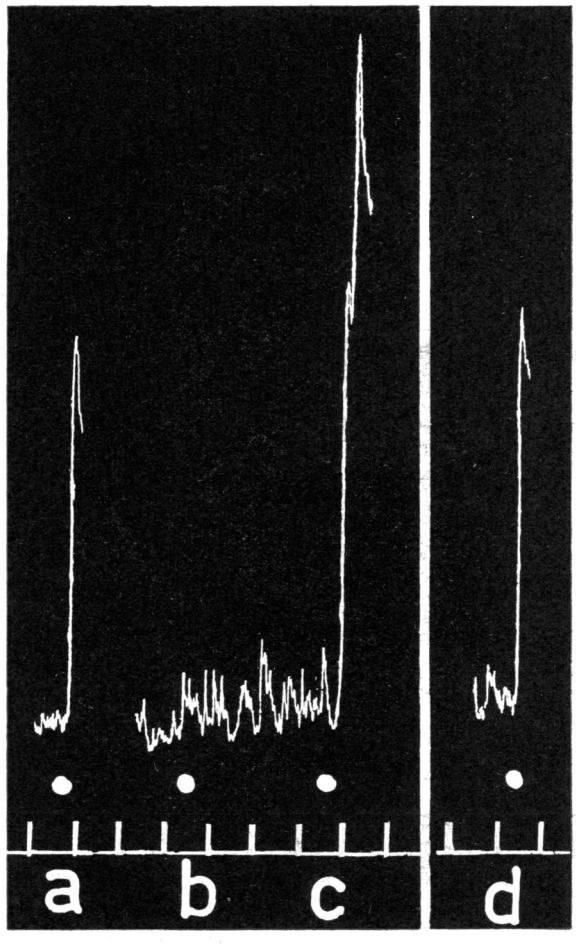

Fig. 9. Enhancement of effect of acetylcholine by small dose of nitromethane. Fresh ileum. *a*, Acetylcholine, 2 ng/ml.; after 3 washings, at *b*, nitromethane, 1 : 5,000. No direct effect on ileum, except enlarged peristaltic movements. *c*, 3 min later, without washing, acetylcholine, 2 ng/ml. Amplitude of contraction about 45% higher than in *a*. *d*, After 3 washings, acetylcholine, 2 ng/ml. Effect similar to *a*.

Mechanism of inhibition by nitroparaffins. The antagonism of nitroparaffins to smooth muscle stimulants is most striking with acetylcholine. As shown in Fig. 11, the inhibition is competitive, since a given concentration of nitroethane is less effective against higher doses of acetylcholine. Therefore, the I 50 values in Table 1 have been determined against a standard concentration of acetylcholine (4 ng/ml.). When the logarithm of I 50 is plotted as function of n, the number of carbon atoms in the normal chain, a straight line is obtained (Fig. 12). Assuming that the I 50 values are proportional to the inhibition constants K_I, the slope of the curve in Fig. 12 measures the free energy change $\triangle F_o$, connected with the transfer of a CH_2-group from the solution to the receptor site. The value of 510 cal per -CH_2-

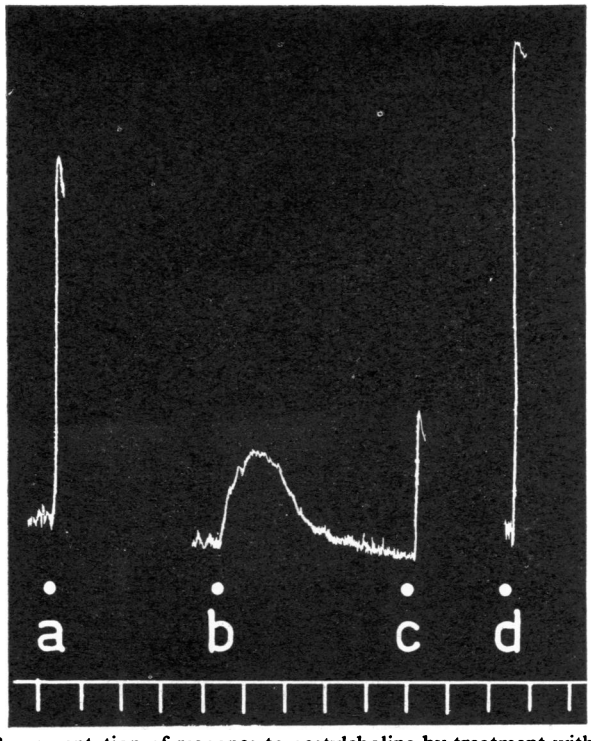

Fig. 10. " Late " augmentation of response to acetylcholine by treatment with nitrocyclopentane. Fresh ileum. *a*, Acetylcholine, 4 ng/ml.; *b*, nitrocyclopentane, 1 : 2,000; contraction and return to initial tonus. *c*, After 5 min, without washing, acetylcholine, 4 ng/ml. Note 60% inhibition. Between *c* and *d*, 2 washings. *d*, Acetylcholine, 4 ng/ml. Note 35% higher peak of contraction as compared to *a*.

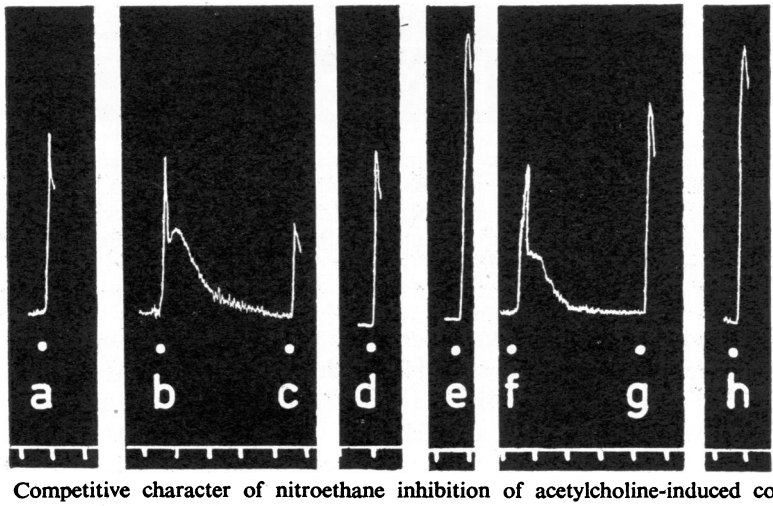

Fig. 11. Competitive character of nitroethane inhibition of acetylcholine-induced contraction. Fresh ileum; between sections 3 washings. *a*, Acetylcholine, 0·4 ng/ml.; *b*, nitroethane, 1 : 1,750; *c*, 4 min later, without washing, acetylcholine, 0·4 ng/ml. Contraction is reduced by 50%. *d*, Acetylcholine, 0·4 ng/ml. Complete recovery after washing out the nitroparaffin. *e*, Acetylcholine, 1·6 ng/ml. ; *f*, nitroethane, 1 : 1,750; *g*, 4 min later, without washing, acetylcholine, 1·6 ng/ml. Contraction reduced by 25%. *h*, Acetylcholine, 1·6 ng/ml.; recovery after removal of nitroparaffin.

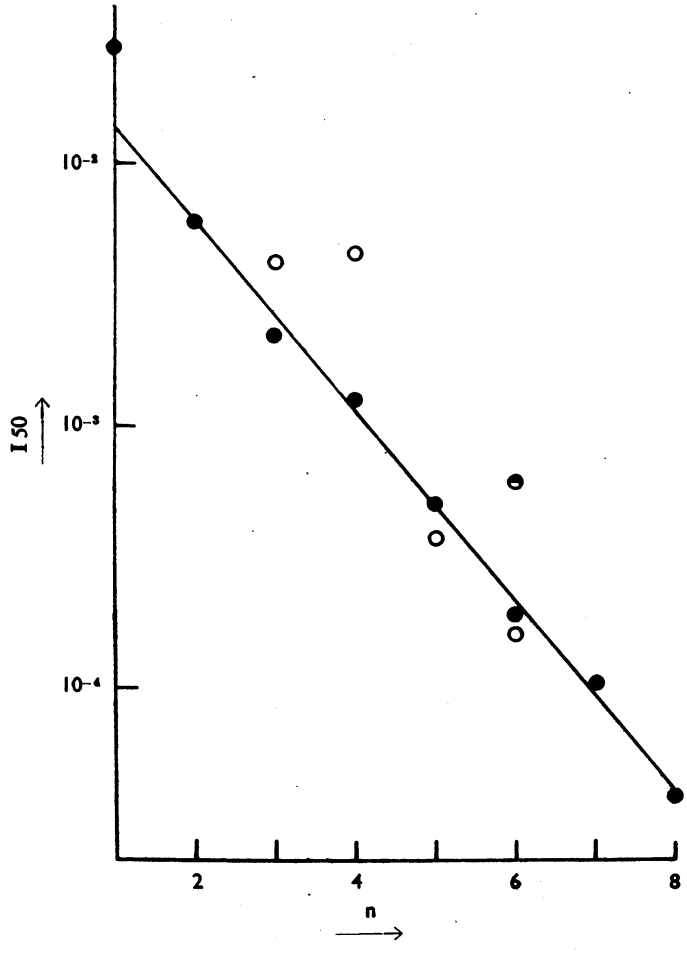

Fig. 12. Inhibitory effect of straight-chain nitroparaffins on acetylcholine-induced contractions as function of n, the number of carbon atoms. Ordinate: log I 50 (the concentration reducing to one-half the contraction provoked by 4 ng/ml. acetylcholine). All experimental points were determined on a single loop of fresh guinea-pig ileum. Between two applications, the loop was incubated with 20 ng/ml. acetylcholine for 5 min. Note that the point for nitromethane (n=1) falls outside the curve. The regression line was calculated only for the points n=2 to n=8 and is represented by the equation: log I 50= −0·358 n −1·524. Open circle at n=3 represents 2-nitropropane; at n=4, 2-nitro-2-methylpropane; at n=5, nitrocyclopentane; at n=6, nitrocyclohexane; half-open circle at n=6 denotes nitrobenzene.

group at 37° C comes so close to the value obtained for the corresponding process of shifting a CH_2-group from the medium to the active surface of pseudocholinesterase (Bergmann & Segal, 1954) as to suggest related mechanisms such as adsorption.

The relationship of nicotine to nitroparaffins is more complex. The latter counteract the excitatory effect of the alkaloid in two ways; by exhausting the acetylcholine reserves of the nerve terminals (see Fig. 7), thus making them less responsive to

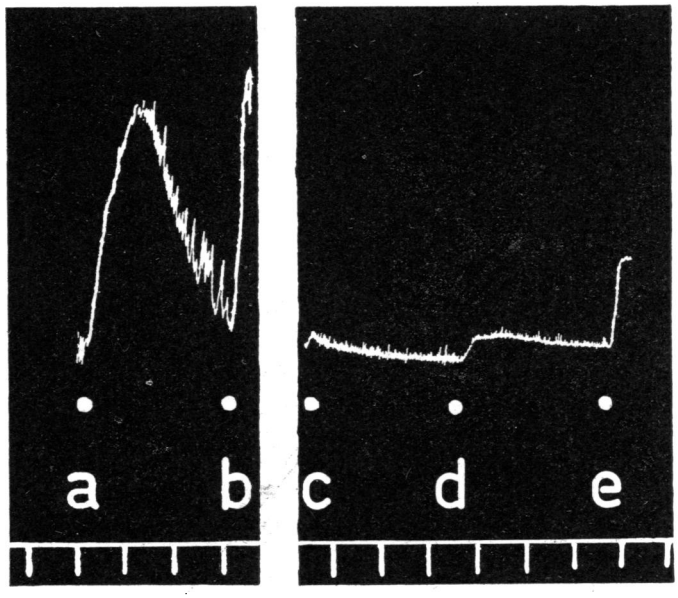

Fig. 13. Nitrobutane blocks the effect of nicotine more efficiently than that of acetylcholine. *a*, Nicotine, 0·6 μg/ml. ; *b*, 3 min later, without washing, acetylcholine, 6 ng/ml.; *c*, 1-nitro-n-butane, 1 : 3,000 ; at *d*, after 3 min incubation, without washing, addition of nicotine, 0·6 μg/ml.; and at *e*, of acetylcholine, 6 ng/ml. Response to nicotine is reduced by 90% and to acetylcholine only by 66%.

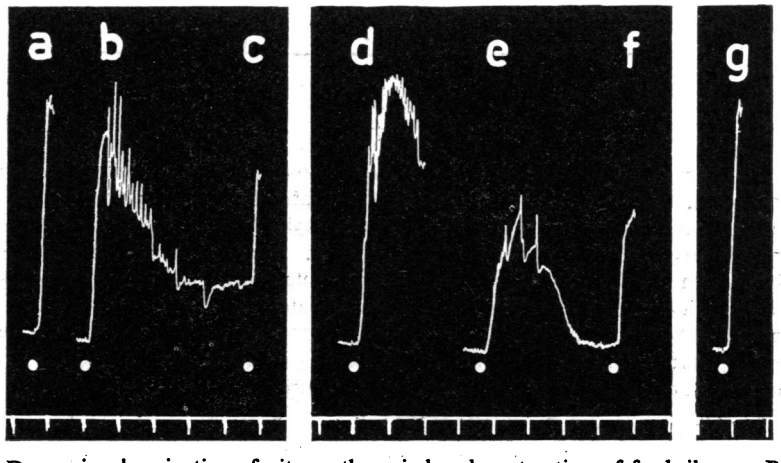

Fig. 14. Depression by nicotine of nitromethane-induced contraction of fresh ileum. Between *a* and *b*, *c* and *d*, *d* and *e*, *f* and *g*, 3 washings each. Dose of acetylcholine, 1 ng/ml. in all experiments. *a*, Acetylcholine; *b*, nitromethane, 1 : 500. Note non-sustained response and large peristaltic waves at height of contraction. *c*, 5 min later, acetylcholine; *d*, nicotine, 0·8 μg/ml.; *e*, nitromethane, 1 : 500. Shape of curve similar to *b*, but amplitude 40% smaller. *f*, Acetylcholine; *g*, recovery of response to acetylcholine.

ganglionic stimulation, and by blocking the action of the liberated transmitter on the muscle fibre. Therefore, the nitroparaffins are more powerful inhibitors of nicotine than acetylcholine action (Fig. 13). On the other hand, nicotine also deprives the nitroparaffins of their substrate for their excitatory action, partly by ganglionic blockade and partly by depletion of the transmitter. Therefore, after a single application of nicotine, the response to nitromethane is greatly reduced (Fig. 14), unless prolonged incubation with acetylcholine replenishes the tissue stores.

The nitroparaffins are less effective against serotonin or bradykinin than against acetylcholine. At their I 50 concentrations, the nitro compounds are inert against

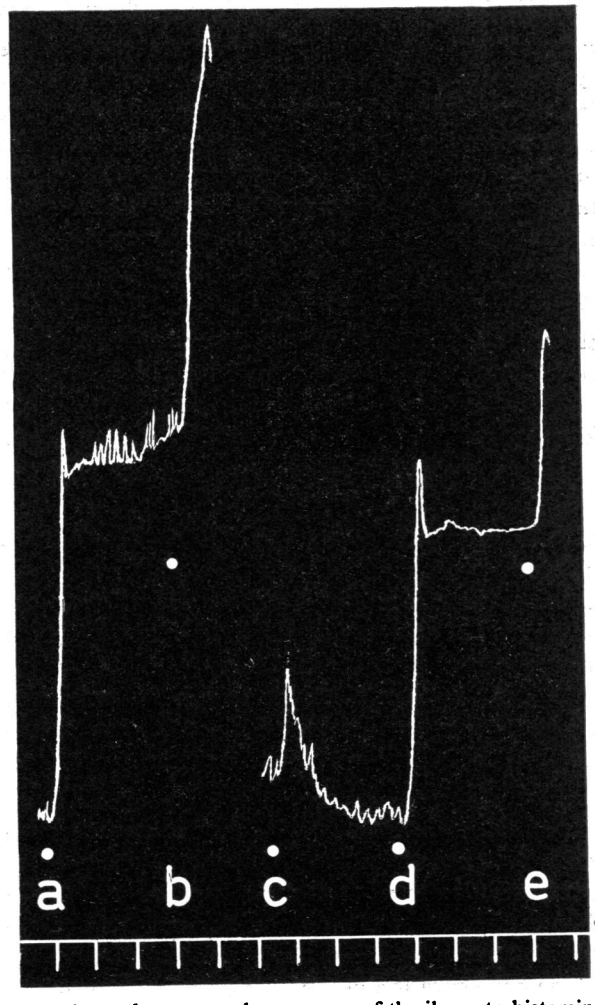

Fig. 15. Influence of 1-nitro-n-butane on the response of the ileum to histamine and acetylcholine. *a*, Histamine, 8 ng/ml. ; *b*, 3 min later, without washing, addition of acetylcholine, 3 ng/ml.; 3 washings, then at *c*, incubation with nitrobutane, 1 : 5,000, for 3 min ; *d* and *e*, addition of histamine and acetylcholine as before. While the response to histamine remains practically unchanged, the amplitude of the contraction to acetylcholine is reduced by 50%.

histamine (Fig. 15). Even when applied in 3 to 4 times higher dosage they reduce the histamine-provoked contraction only by about 20%.

Effect of branched nitroparaffins and of cyclic nitro derivatives. Both 2-nitropropane and nitro-tert. butane were more active as stimulatory agents than their straight-chain analogues, but less effective as acetylcholine antagonists (Table 1). In this respect the tert. butane derivative falls short even of nitroethane.

The 3 cyclic derivatives studied, viz., nitrocyclopentane, nitrocyclohexane and nitrobenzene, show a moderate excitatory action on the smooth muscle. The cyclopentane and the aromatic derivative exceed the inhibitory effect of the straight-chain nitro compounds with the same number of carbon atoms, while nitrocyclohexane exhibits less than half of the antispasmodic activity of the n-hexane derivative.

The use of nitroparaffins for the study of pharmacological mechanisms. The sensitivity of the excitatory effect of nitroparaffins to depletion of the acetylcholine reserves provides a useful procedure to distinguish between direct and indirect actions of smooth muscle stimulants. Any indirect effect must lead to depression of nitroparaffin-induced contraction. By recurrent application of the stimulants involved, it could thus easily be shown that neither bradykinin nor small doses of histamine touch upon the transmitter stores in the nerve endings. On the contrary, repeated doses of bradykinin usually enhanced somewhat the response to nitromethane. This curious phenomenon will be dealt with separately.

The fact that the nitroparaffins liberate acetylcholine from the tissue stores suggested the possibility that other biogenic amines may also be released under the influence of the nitro compounds. However, 30 min incubation of mast cells with nitromethane did not result in the liberation of measurable amounts of histamine.

DISCUSSION

The nitroparaffins act apparently on a multitude of sites. They activate both the ganglionic synapse and the post-ganglionic fibre, as shown by interaction with hexamethonium. The latter, even in very large concentrations, reduces to about half the contraction of the intestinal muscle, caused by a suitable nitroalkane, whereas small doses of morphine suppress this effect completely. The nitroparaffins thus release acetylcholine from the nerve terminals by a dual mechanism producing a profound exhaustion of the transmitter stores. For the same reason, the nitro compounds also serve as a useful tool to demonstrate the replenishment of the tissue stores from an external source.

The ability of the stores in the intestinal wall to pick up acetylcholine quickly from the medium suggests that not all of the physiological transmitter, liberated during a response, is necessarily lost by enzymic destruction, as is claimed in common descriptions of the acetylcholine cycle. Part of the mediator may be reabsorbed by nervous structures for repeated use. As the amount of released mediator is always in large excess of the concentration needed for chemical transmission, re-utilization would improve the economy of transmitter turnover.

The facile release of acetylcholine under the influence of nitroparaffins indicates that these compounds change the permeability of the membrane, covering the nerve

endings. On this basis, the augmentation by subliminal concentrations of nitromethane of contractions induced by acetylcholine (Fig. 9) can be explained as follows: the nitro compound, itself unable to liberate the transmitter, prepares the nerve terminal for the subsequent attack by exogenous acetylcholine. The action of the latter is therefore increased by release of intrinsic mediator. If this explanation is correct, it appears also possible that the excitatory effect of exogenous acetylcholine in general involves participation of released transmitter.

Changes in the presynaptic endings, produced by electrical volleys, have previously been held responsible for facilitation of synaptic transmission, whether in response to electrical shocks (Larrabee & Bronk, 1947) or to chemical stimulants (Volle, 1961). Subliminal doses of the nitroparaffins appear to accomplish the same effect by "chemical facilitation."

The biphasic effect of the nitroparaffins at the neuromuscular junction, viz., release of transmitter followed by blockade of the post-synaptic membrane, suggests a similar sequence at the ganglionic synapse. Therefore, the ganglionic excitation, demonstrated for nitroparaffins in Fig. 6, probably represents also a liberation of transmitter from the *presynaptic* terminals. On the other hand, one component of the very effective antagonism of the nitro compounds to nicotine may be represented by blockade of the *postsynaptic* membrane in the ganglion.

The most interesting aspect of nitroparaffin action is undoubtedly the opposite influence on pre- and post-ganglionic membranes. The inhibitory action resembles in certain respects that of atropine. Polar molecules arrange themselves on the surface with their hydrophilic end facing outwards, while the aliphatic chain enters the lipoid layer, thus enhancing internal pressure (Skou, 1954a, b). Therefore, the inhibitory power is increased by lengthening the chain, but is diminished by branching.

On the other hand, the release of transmitter from the nerve endings requires an increase in permeability of the presynaptic membrane. It is conceivable that here the molecules are oriented in the reverse fashion, since branching of the chain augments the stimulatory action.

The effects of nitroparaffins elucidated by the present studies should not be confined to the intestinal wall. Actions on other autonomous organs are to be expected. Experiments, pertinent to this aspect of the pharmacology of aliphatic nitro compounds, will be reported in due course.

Finally, as mentioned in the introduction, the nitroparaffins were chosen because of their large dipole moment, which approaches the value of about 4.0 for the higher members of the series (Groves & Sugden, 1935). If this is indeed a physical property, connected with the biological effects observed, other groups with similar high polarity, such as organic nitriles, should reveal analogous actions. Studies in this direction are now being undertaken.

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