RESULTS

RAT ILEUM

Sympathomimetic effects of nicotine, DMPP and ACh on the isolated rat ileum

In the absence of hyoscine, nicotine (0.25 - 4 µg/ml) and DMPP (0.25 - 4 µg/ml) produced dose-related relaxant responses but ACh (1 - 8 µg/ml) produced dose-related contractions. In the presence of hyoscine, all the three drugs produced dose-related relaxations of the rat ileum for 3 - 4 hours (Fig. 2). In subsequent experiments, unless otherwise stated, nicotine and DMPP were used in a concentration of 2 µg/ml and the concentration of ACh was 4 µg/ml. These doses produced 50 to 61% of their own maximum responses.

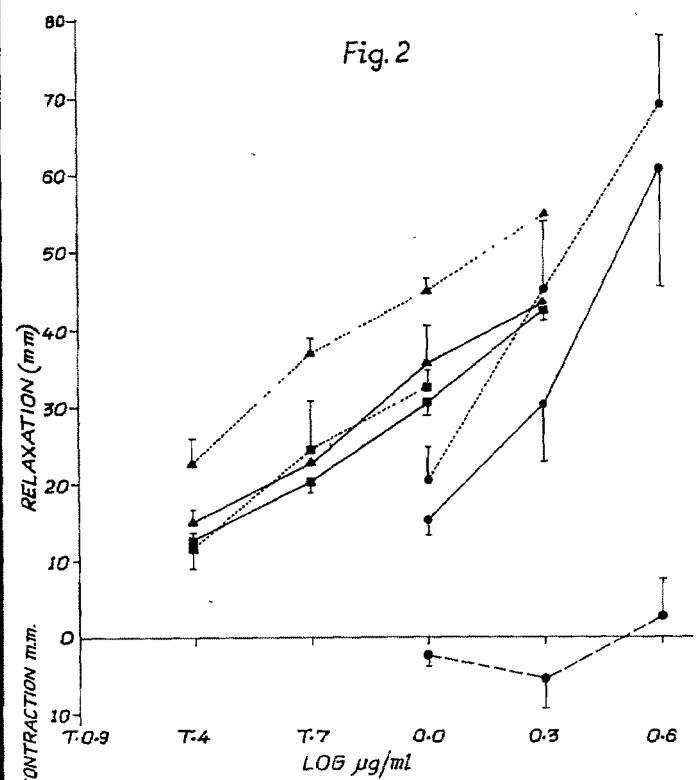
Effect of Ca^{++} -deprivation

After eliciting control responses in the presence of normal Tyrode solution, the preparations were exposed to Ca^{++} -free Tyrode solution containing sodium edetate (30 µg/ml) for 30 minutes. Following this, responses to

Fig. 2

Isolated rat ileum (suspended in Tyrode solution containing hyoscine 1 µg/ml). Dose-response curves for the effects of nicotine, (▲) DMPP (■), and ACh (●). Responses represented by continuous lines were obtained in the presence of normal Tyrode solution. In the presence of Ca^{++} -free solution, nicotine and DMPP were inactive and ACh produced responses shown by ~~dotted~~ broken line, when these preparation were bathed with normal Tyrode solution, responses shown by ~~broken~~ dotted lines were obtained. Vertical lines indicate S.E. of means (4 observations).

Fig. 2


80
70
60
50
40
30
20
10
0
10
RELAXATION (mm)
CONTRACTION m.m.
T·0·9
T·4
T·7
0·0
0·3
0·6
LOG µg/ml

nicotine, DMPP and ACh were reelicited. Nicotine and DMPP had no effect in Ca^{++} - free solution (6 experiments). ACh produced either relaxation (3 experiments) or slight contraction (2 experiments) or no effect (2 experiments). When these preparations were bathed in normal Tyrode solution, it was possible to obtain full dose-response curves for relaxation to all the agents. In fact, responses to nicotine and ACh were markedly enhanced (Fig. 2).

Effect of adrenergic neurone blocking agents

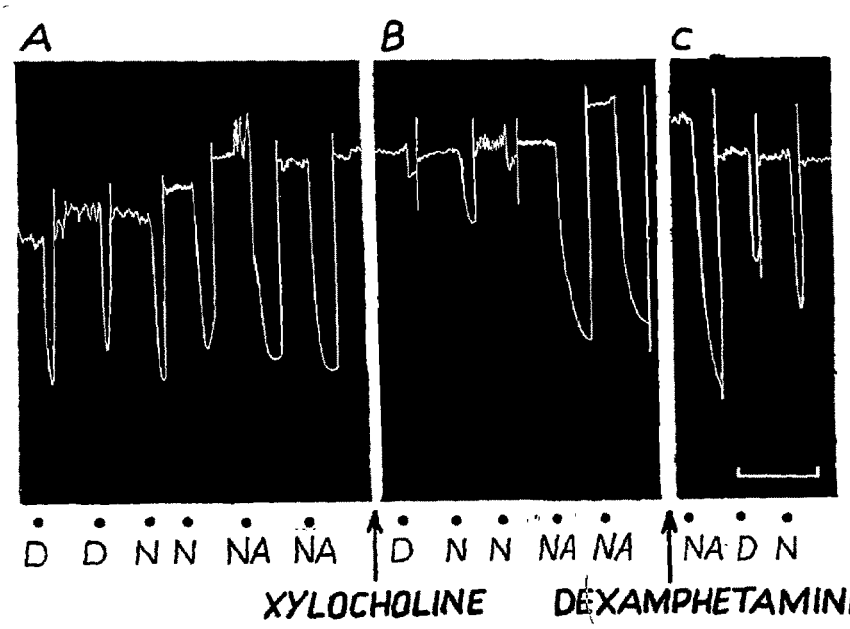
Xylocholine. After eliciting control responses to nicotine, DMPP, ACh and NA, the preparation was exposed to xylocholine (20 µg/ml; 6 experiments) for 20 minutes. Xylocholine completely blocked relaxant responses to nicotine and DMPP in 3 experiments and considerably reduced them in 3 others. ACh-induced responses were completely blocked in 2 experiments and considerably reduced in 4 experiments.

In all experiments, responses to NA either remained unaffected or were slightly potentiated (Table 1; Fig. 3).

Fig. 3

Isolated rat ileum (suspended in Tyrode solution containing hyoscine, 1 µg/ml). Responses to DMPP (2 µg/ml at D), nicotine (2 µg/ml at N) and NA (10 ng/ml at NA). Panel A shows control responses. Between panels A and B, the preparation was exposed to xylocholine (20 µg/ml) for 20 min and washed. Panel B shows response in presence of xylocholine (20 µg/ml). Between panels B and C, the preparation was washed and then exposed to dexamphetamine (10 µg/ml) for 10 min and washed. Panel C shows responses in presence of dexamphetamine (10 µg/ml).
Time mark, 1 minute.

Fig. 3



In 4 experiments, the blocking action of xylocholine could be reversed by washing out the drug; in two experiments the block lasted for 10 - 15 min after removal of the drug from the bath.

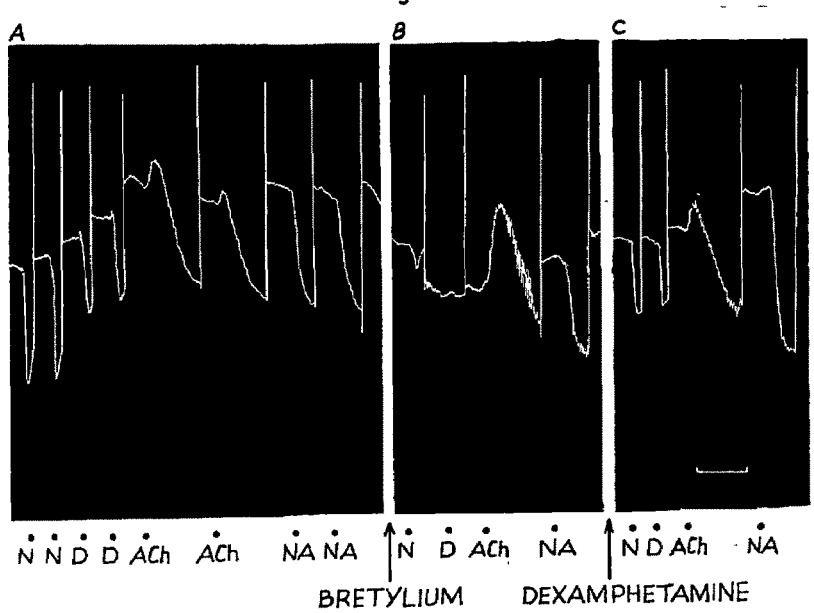
Bretylium. After a 20 min exposure to bretylium (20 µg/ml; 8 experiments) responses of rat ileum to nicotine and DMPP were either totally blocked (4 experiments) or considerably reduced (4 experiments). Similarly, responses to ACh were either totally blocked (2 experiments), or considerably reduced (6 experiments). Responses to NA were either potentiated (4 experiments) or unaffected (4 experiments) (Table 1, Fig. 4). When the block was complete, the effect persisted for 10 - 15 min after washing. When the block was partial, the effect was immediately restored on washing.

Guanethidine. Guanethidine (20 µg/ml for 20 min; 6 experiments) either totally blocked responses to nicotine and DMPP (3 experiments) or considerably reduced them (3 experiments). Responses to ACh were either totally blocked (2 experiments) or considerably reduced (4 experiments).

Fig. 4

Isolated rat ileum (suspended in Tyrode solution containing hyoscine, 1 μg/ml). Responses to nicotine (2 μg/ml at N), DMPP (2 μg/ml at D), ACh (4 μg/ml at ACh), and NA (10 ng/ml at NA). Panel A shows control responses. Between panels A and B, the preparation was exposed to bretylium (20 μg/ml) for 20 min and washed. Panel B shows responses in the presence of bretylium (20 μg/ml). Between panels B and C, the preparation was washed and then exposed to dexamphetamine (10 μg/ml) for 10 min and washed. Panel C shows responses in the presence of dexamphetamine (10 μg/ml). Time mark, 1 minute.

Fig. 4


A
B
C
N N D D ACh ACh NA NA
N D ACh NA
N D ACh NA
BRETYLIUM
DEXAMPHETAMINE

Responses to NA were either potentiated (4 experiments) or unaffected (2 experiments) (Table 1; Fig. 5). The blocking action of guanethidine persisted for 60 - 90 min despite repeated washing.

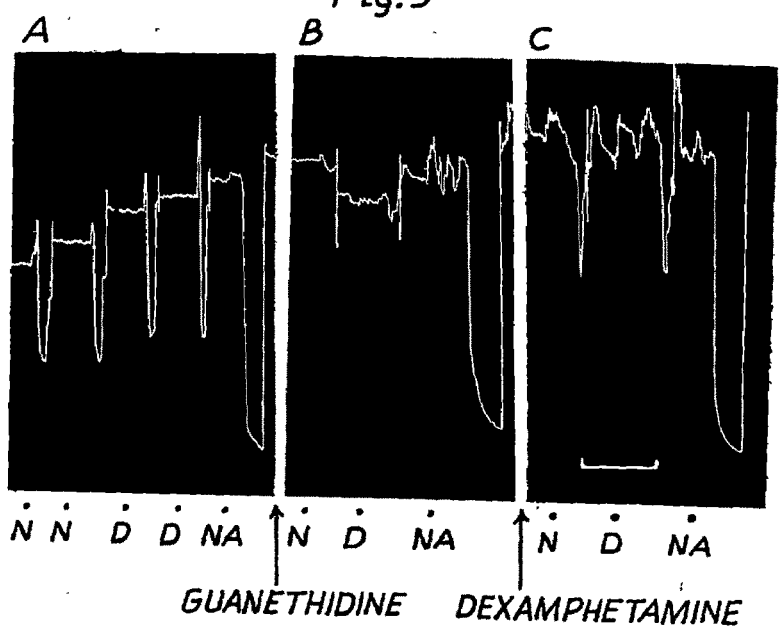
Reversal of the blocking action of xylocholine, bretylium and guanethidine by dexamphetamine. Dexamphetamine, methylamphetamine and certain indirectly acting sympathomimetic amines are capable of reversing the adrenergic neurone blocking action of bretylium, guanethidine and xylocholine (Day and Rand, 1962; Day, 1962; Gokhale et al, 1965, 1966). It was, therefore, of interest to see if a similar reversal of the block of effects of nicotine, DMPP and ACh by bretylium, guanethidine or xylocholine, occured following dexamphetamine.

The preparations were exposed to xylocholine (20 µg/ml) or bretylium (20 µg/ml) or guanethidine (20 µg/ml) for 20 min, after which they were washed. The blocking drug was again added and after 6 min, responses to nicotine, DMPP and ACh were elicited and found blocked. This was followed by the addition of dexamphetamine (10 µg/ml) and redetermination 10 min later of responses to nicotine, DMPP and ACh in

Fig. 5

Isolated rat ileum (suspended in Tyrode solution containing hyoscine 1 μg/ml). Responses to nicotine (2 μg/ml at N), DMPP (2 μg/ml at D), and NA (10 ng/ml at NA). Panel A shows control responses and panel B shows responses after the preparation was exposed to guanethidine (20 μg/ml) for 20 min and washed. Between panels B and C the preparation was exposed to dexamphetamine (10 μg/ml) for 10 min and washed. Panel C shows responses in the presence of dexamphetamine (10 μg/ml). Time mark, 1 minute.

Fig.5



the presence of dexamphetamine.

Dexamphetamine totally reversed (Fig. 3) the blocking effect of xylocholine on nicotine and DMPP-induced relaxant responses in 2 experiments and did not have any effect in 4 experiments. Dexamphetamine reversed either totally (2 experiments) or partially(2 experiments) the blocking effect of xylocholine on ACh-induced relaxant responses or had no effect (1 experiment).

Dexamphetamine restored partially the relaxant effect blocked by bretylium in 1 of the 7 experiments with nicotine and DMPP and 3 of the 7 experiments with ACh (Fig. 4).

Dexamphetamine could completely restore (Fig. 5) the relaxant effects of nicotine and DMPP blocked by guanethidine in only 1 of the 5 experiments. In the case of ACh, partial reversal of the blocking action of guanethidine was observed in two out of 5 experiments.

Effect of pre-treatment with reserpine

In preparations from rats pretreated with reserpine, nicotine and DMPP did not produce relaxation (6 experiments)

and ACh either did not produce relaxation (5 experiments) or produced contraction (1 experiment). After initial testing of drug effects, the preparations were exposed to NA (2 µg/ml) for a period of 30 minutes. The NA was then removed and the preparations repeatedly washed for 20 minutes. Nicotine, DMPP and ACh now produced relaxant effects comparable to those obtained in control preparations. Exposure of preparations to xylocholine (20 µg/ml for 20 min; 2 experiments), bretylium (20 µg/ml for 20 min; 2 experiments) or guanethidine (20 µg/ml for 20 min; 2 experiments) led to total block of response restituted by NA. Following wash, the preparations were exposed to dexamphetamine (10 µg/ml for 10 min). There was 28 - 50% reversal of the block produced by bretylium (1 experiment) and xylocholine (2 experiments). The block produced by guanethidine was not revered. The results of typical experiments are shown in Fig. 6.

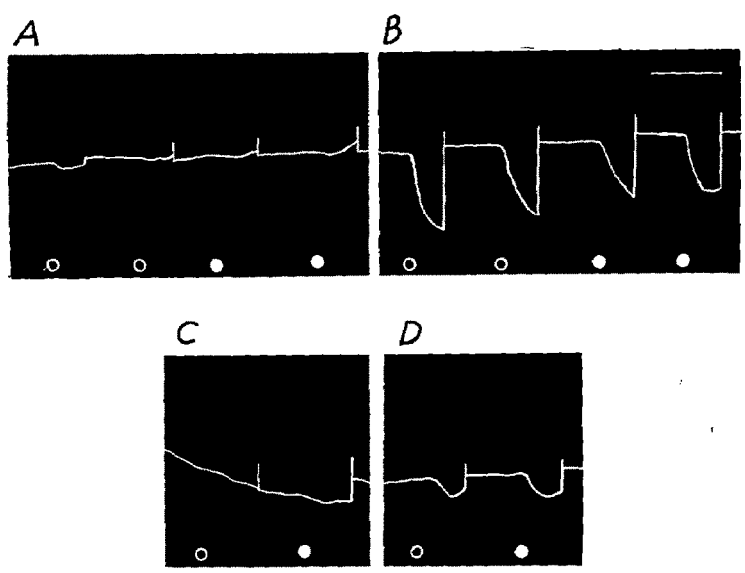
Effect of alpha- and beta-adrenoceptor blockers

Pronethalol (10 µg/ml for 10 min; 5 experiments) or phentolamine (5 µg/ml for 5 min; 3 experiments) was added

Fig. 6

Isolated ileum (suspended in Tyrode solution containing hyoscine 1 μg/ml) from reserpinized rat. Open and closed circles represent respectively responses to nicotine (2 μg/ml) and DMPP (2 μg/ml). Panel A shows control responses, panel B shows responses after the preparation was exposed to NA (2 μg/ml) for 30 min and then repeatedly washed for 20 minutes. Panel C shows responses after bretylium (20 μg/ml) was kept in the bath for 20 minutes. Following wash, the preparation was exposed to dexamphetamine (10 μg/ml) for 10 min and responses shown in panel D were obtained. Time mark, 1 minute.

Fig. 6



to bath before exposure to nicotine, DMPP and ACh. Pronethalol or phentolamine only partially blocked the relaxant responses to nicotine, DMPP, ACh and NA. However, simultaneous exposure of the preparations to both phentolamine and pronethalol resulted in a total block of responses to all the drugs (Table 1; Fig. 7).

Effect of ganglion blocking agents

Hexamethonium (10 µg/ml for 10 min; 6 experiments), pempidine (5 µg/ml for 10 min; 3 experiments) and mecamylamine (5 µg/ml for 10 min; 4 experiments) produced substantial to complete block of relaxant responses to nicotine and DMPP, but either did not affect or potentiated responses to ACh and NA (Table 1, Fig. 8).

Effect of cocaine, DMI and procaine

Cocaine (10 µg/ml for 10 min; 6 experiments), DMI (5 µg/ml for 10 min; 6 experiments)and procaine (5 µg/ml for 10 min; 6 experiments) either totally blocked relaxant responses to nicotine and DMPP or considerably reduced them. On the other hand, responses to ACh and NA were

Fig. 7

Isolated rat ileum (suspended in Tyrode solution containing hyoscine 1 µg/ml). Responses to DMPP (2 µg/ml at D) nicotine (2 µg/ml at N), ACh (4 µg/ml at ACh) and NA (10 ng/ml at NA). Panel A shows control responses; panel B shows responses after the addition of pronethalol (10 µg/ml for 10 min) to the bath, and panel C shows responses after the addition of pronethalol (10 µg/ml) and phentolamine (5 µg/ml) for 10 minutes.
Time mark, 1 minute.

Fig. 7

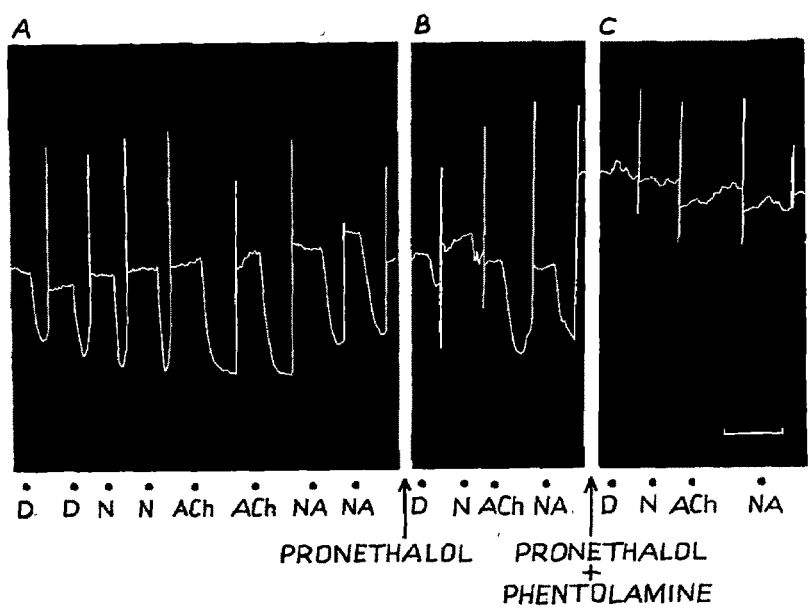

A
B
C
D D N N ACh ACh NA NA D N ACh NA D N ACh NA
PRONETHALOL
PRONETHALOL
+
PHENTOLAMINE

Fig. 8

Isolated rat ileum preparations (suspended in Tyrode solution containing hyoscine, 1 μg/ml). Responses to nicotine (2 μg/ml at N), DMPP (2 μg/ml at D), NA (10 ng/ml at NA) and ACh (4 μg/ml at ACh). Panel A shows control responses. Between panels A and B, the preparations were exposed to hexamethonium (10 μg/ml for 10 min) in (a), to pempidine (5 μg/ml for 10 min) in (b) and to mecamylamine (5 μg/ml for 10 min) in (c) and washed. Panel B shows responses in presence of the ganglion blocker. Time mark, 1 minute.

Fig. 8a

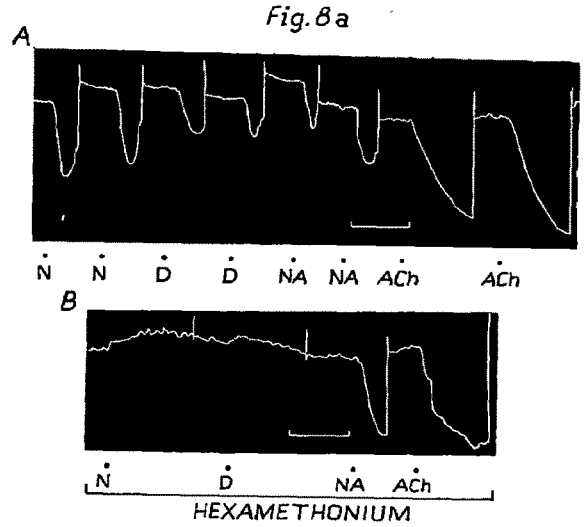


Fig. 8b

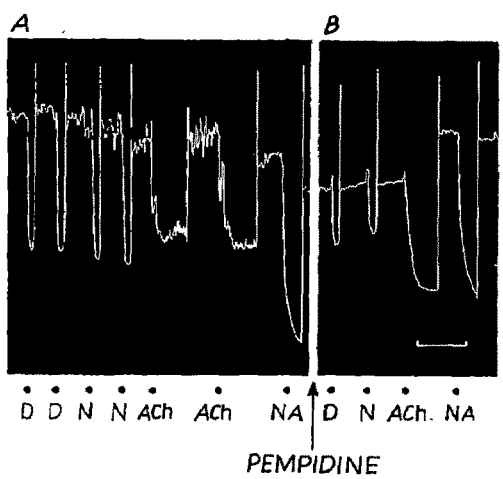
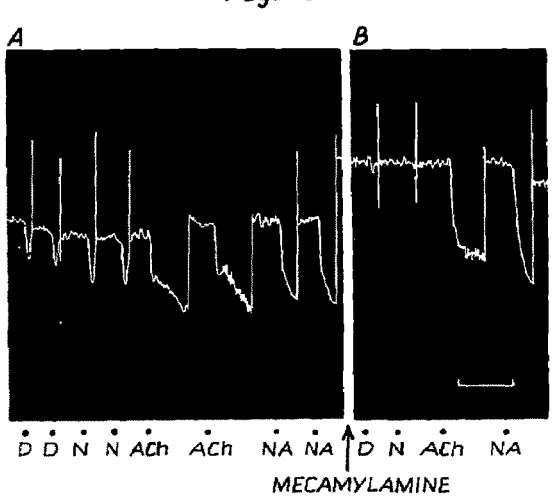


Fig. 8c



markedly potentiated (Table 1, Fig. 9).

Effect of tetrodotoxin

Tetrodotoxin (.5 ng/ml for 30 min; 4 experiments) markedly inhibited responses to nicotine and DMPP, did not affect those to ACh and markedly potentiated those to NA (Table 1, Fig. 10).

Effect of low temperature

After control relaxant responses to nicotine , DMPP, ACh and NA were elicited at 35°C the bath fluid temperature was lowered to 18°C (4 experiments). Responses to nicotine and DMPP were totally blocked, whereas those to ACh were unaffected or slightly reduced and those to NA were potentiated (Table 1, Fig. 11).

Effect of HC-3 and triethylcholine

The relaxant responses to nicotine and DMPP were markedly inhibited by HC-3 (6 experiments) and triethylcholine (5 experiments). However, responses to ACh and NA

Fig. 9

Isolated rat ileum preparations suspended in Tyrode solution containing hyoscine (1 μg/ml). Responses to nicotine (2 μg/ml at N), DMPP (2 μg/ml at D), ACh (4 μg/ml at ACh) and NA (10 ng/ml at NA). Panel A shows control responses. Between panels A and B, the preparations were exposed to cocaine (10 μg/ml for 10 min) in (a), to procaine (5 μg/ml for 10 min) in (b) and to DMI (5 μg/ml for 10 min) in (c) and washed. Panel B shows responses in the presence of the blocking agent.

Time mark, 1 minute.

Fig. 10

Isolated rat ileum (suspended in Tyrode solution containing hyoscine 1 µg/ml). Responses to DMPP (1 µg/ml at D), nicotine (1 µg/ml at N), ACh (4 µg/ml at ACh) and NA (10 ng/ml at NA). Panel A shows control responses. Between panels A and B, the preparation was exposed to tetrodotoxin (5 ng/ml) for 30 min and washed and responses shown in panel B were elicited.

Time mark, 1 minute.

Fig. 10

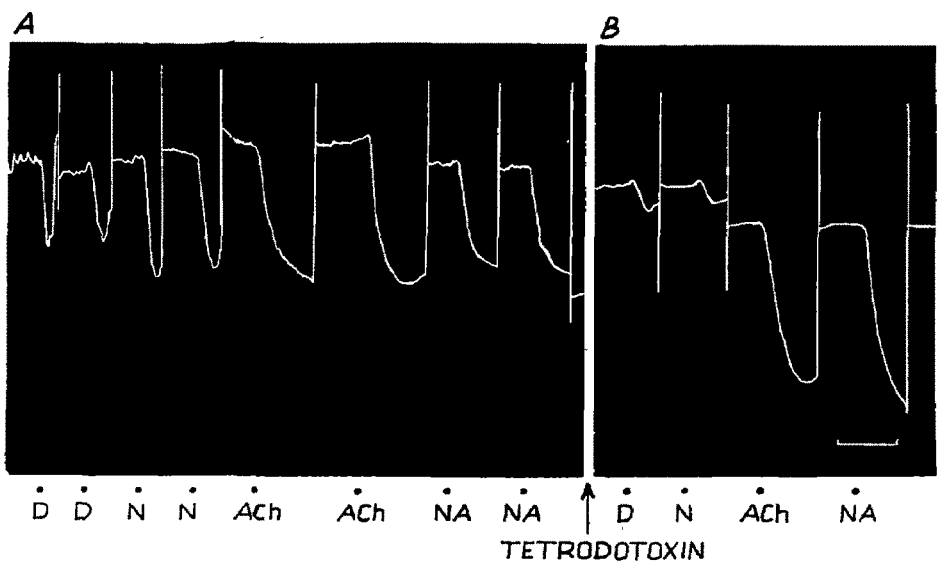
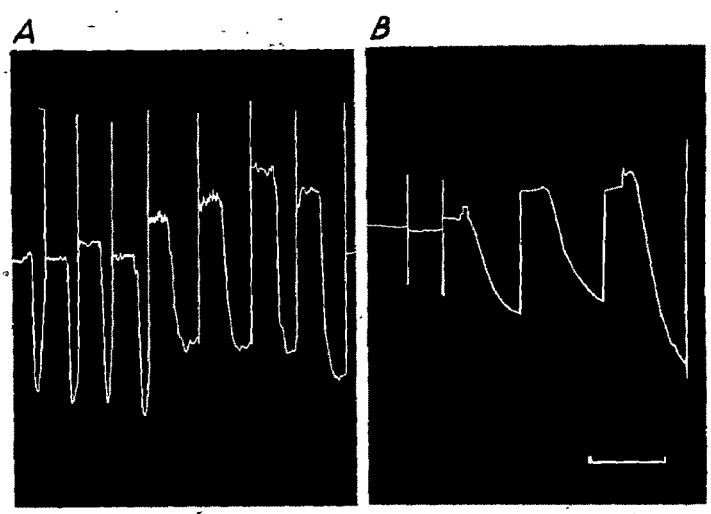


Fig. 11

Isolated rat ileum (suspended in Tyrode solution containing hyoscine 1 µg/ml). Responses to DMPP (1 µg/ml at D), nicotine (1 µg/ml at N), ACh (4 ug/ml at ACh) and NA (10 ng/ml at NA). Panel A shows control responses at 35°C and panel B shows responses at 18°C.

Time mark, 1 minute.

Fig. 11



were potentiated by both HC-3 and triethylcholine (Table 1). Choline (100 μg - 2 mg/ml for 30 min) did not reverse the blocking action of HC-3 and triethylcholine on relaxant responses to nicotine and DMPP (Fig. 12).

Effect of HC-3, triethylcholine and adrenergic neurone blockers on the release of ACh by nicotine and DMPP

Nicotine (1 μg/ml) and DMPP (1 μg/ml) released 17.2 $\pm$ 2.1 ng/g/min and 22.1 $\pm$ 6.7 ng/g/min respectively of ACh. The release was significantly ($P < 0.01$) reduced by HC-3 and guanethidine but not ($P > 0.05$) by triethylcholine and bretylium (Fig. 13). Since the tissue was incubated with HC-3 and triethylcholine for 90 min, it was necessary to determine the control release of ACh after incubation in Tyrode solution for 90 minutes. The control release at the end of 90 min was not significantly different ($P > 0.05$) from that in the beginning of the experiment.

Fig. 12

Isolated rat ileum preparations (suspended in Tyrode solution containing hyoscine 1 μg/ml). Responses to nicotine (1 μg/ml at N), ACh (4 μg/ml at ACh) and NA (10 ng/ml at NA). Panel A shows control responses. Between panels A and B, the preparations were exposed to HC-3 (60 μg/ml) in (a) and triethylcholine (110 μg/ml) in (b), three times at 30 min intervals and washed and responses shown in panel B were elicited. Between panels B and C, the preparations were exposed to choline (1 mg/ml) for 30 min and washed and responses shown in panel C were elicited.
Time mark, 1 minute.

Fig. 12a

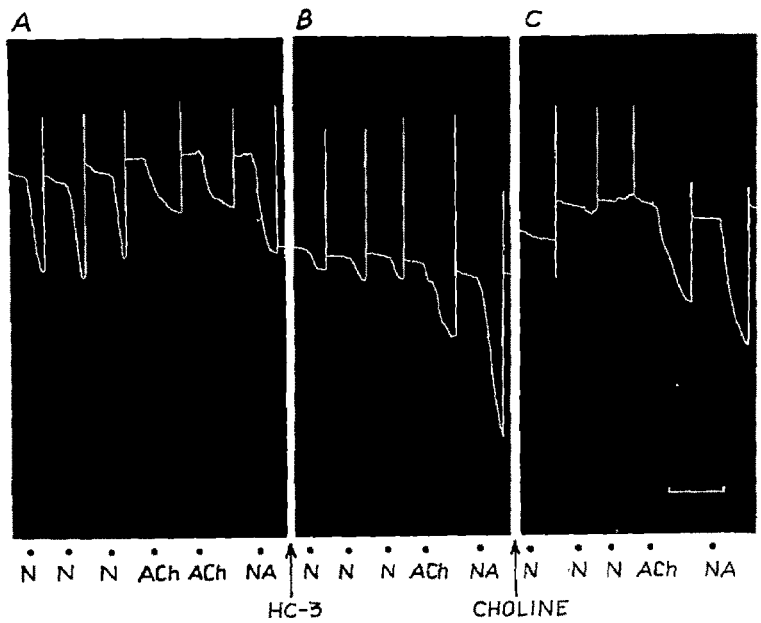


Fig. 12 b

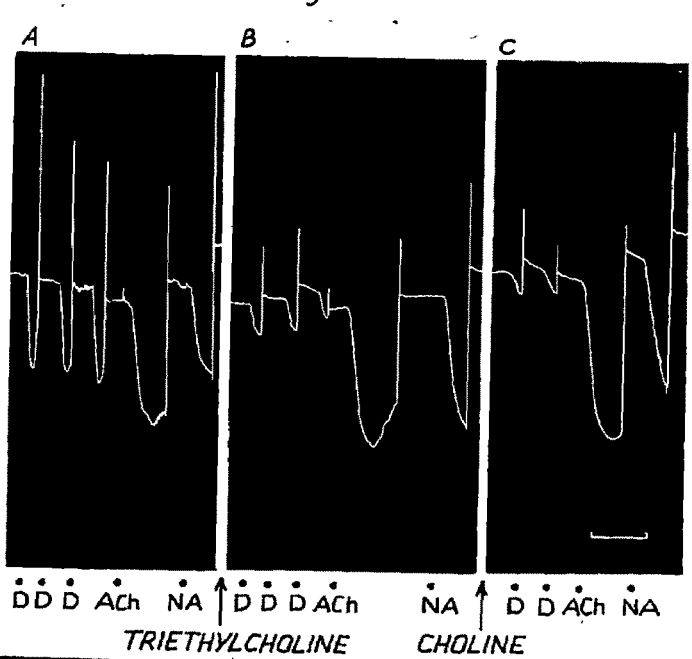


Fig. 13

Effect of nicotine (a) and DMPP (b) on the release of ACh from isolated rat ileum preparations suspended in Tyrode solution. ACh was assayed on the superfused isolated guinea pig ileum. White bars depict control release. Vertical bars stippled bars, horizontal bars and black bars depict the influence on release respectively of HC-3, guanathidine, triethylcholine and bretylium. Vertical lines represent S.E. of means (4 observations).

Fig. 13

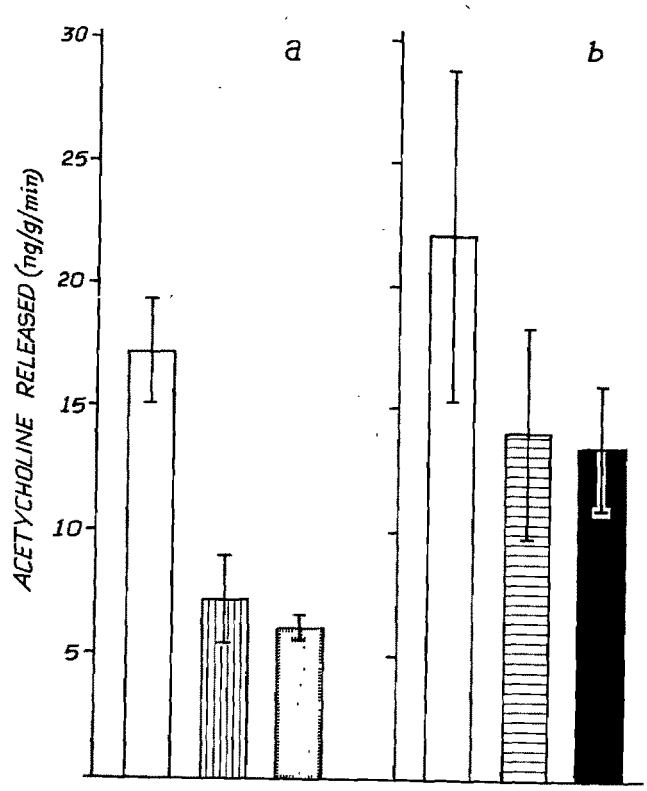


TABLE - 1

Modification by various treatments or procedures of the relaxant responses of rat isolated ileum bathed with Tyrode solution containing hyoscine (1 μg/ml) to nicotine, DMPP, ACh and NA.

		Mean response* ± S.E.M.			
		Nicotine	DMPP	ACh	NA
Xylocholine 20 μg/ml for 20 min	(6)	36.2 ± 2.3	32.5 ± 5.06	61.2 ± 12.1	133.8 ± 15.35
Bretylium 20 μg/ml for 20 min	(8)	19.4 ± 9.2	17.6 ± 9.20	41.4 ± 8.70	121.0 ± 17.90
Guanethidine 20 μg/ml for 20 min	(6)	10.0 ± 8.2	10.0 ± 9.60	17.4 ± 17.40	129.9 ± 17.30
Pronethalol 10 μg/ml for 10 min	(5)	34.0 ± 3.7	40.0 ± 5.10	57.4 ± 9.20	51.8 ± 9.50
Phentolamine 5 μg/ml for 5 min	(3)	36.0 ± 6.3	36.3 ± 8.60	34.6 ± 4.60	54.3 ± 7.30
Pronethalol and Phentolamine for 10 min	(5)	0	0	0	0
Hexamethonium 10 μg/ml for 10 min	(6)	21.5 ± 8.4	12.5 ± 6.10	106.0 ± 5.20	115.0 ± 9.72
Pempidine 5 μg/ml for 10 min	(3)	2.7 ± 2.7	6.5 ± 6.23	136.2 ± 19.40	137.0 ± 19.00
Mecamylamine 5 μg/ml for 10 min	(4)	0	0	134.3 ± 7.80	143.3 ± 18.60
Cocaine 10 μg/ml for 10 min	(6)	9.0 ± 4.2	14.0 ± 6.50	138.3 ± 10.80	152.0 ± 8.60
DMI 5 μg/ml for 10 min	(6)	0	0	138.2 ± 14.20	133.8 ± 14.80
Procaine 5 μg/ml for 10 min	(4)	23.8 ± 4.1	29.5 ± 0.60	163.5 ± 13.30	126.0 ± 6.00
Tetrodotoxin 5 ng/ml for 30 min	(4)	16.0 ± 5.7	23.25± 8.00	112.3 ± 8.50	142.5 ± 13.60
Low temperature 18°C for 20 min	(4)	0	0	93.9 ± 6.00	118.3 ± 3.50
HC-3	(6)	29.1 ± 1.8	21.3 ± 7.80	122.6 ± 10.70	108.6 ± 5.50
Triethylcholine	(5)	33.8 ± 0.9	27.8 ± 3.20	121.8 ± 5.20	133.2 ± 7.80

* Expressed as percentage of the initial control response.

Doses of ACh and NA were 4 μg/ml and 10 ng/ml respectively and those of nicotine and DMPP were 2 μg/ml except in experiments with tetrodotoxin, low temperature, HC-3 and triethylcholine where 1 μg/ml doses were employed. For doses and contact time of HC-3 and triethylcholine, see Methods.

Figures in parentheses indicate the number of experiments.

RABBIT ILEUM

Responses to periarterial nerve stimulation (Finkleman preparation); modification by NA .

1 - 3 days old rabbits

Preparations from 1 day old rabbits always exhibited motor responses to nerve stimulation at all frequencies (Table 2, Fig. 14). Following exposure of the tissue to NA, nerve stimulation failed to evoke any response. NA (200 - 500 ng/ml) did not elicit any relaxant effect on the intestine.

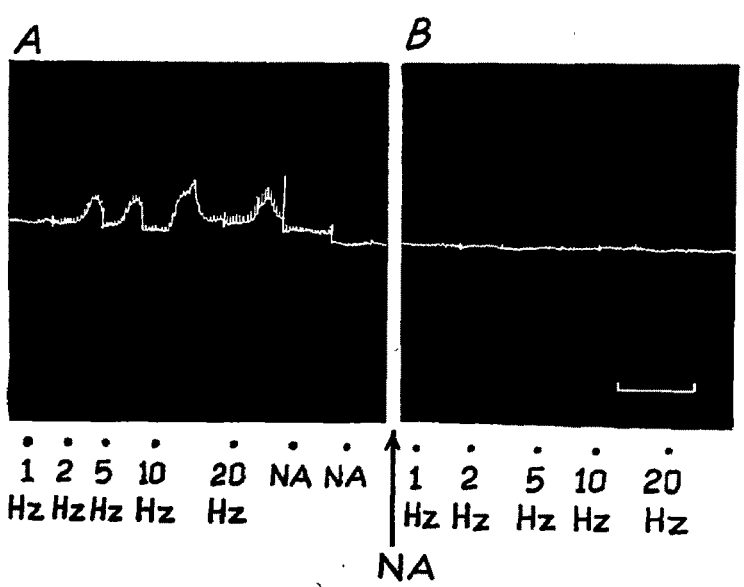
Like preparations from 1 day old rabbits, these preparations also showed contractions in response to nerve stimulation at all frequencies (Table 2, Fig. 15). However, NA could elicit relaxant effects, but generally failed to convert motor responses to relaxant responses. In one, 3 day old rabbit preparation, NA could reverse motor response to relaxant responses at 10 and 20 Hz (Fig. 16).

Fig. 14

Finkleman preparation from 1 day old rabbit (suspended in McEwen's solution). Responses to periaterial nerve stimulation at different frequencies are indicated in Hz. Panel A shows control responses to periarterial nerve stimulation and to NA (200 ng/ml first response and 500 ng/ml second response). Between panels A and B, the preparation was exposed to NA (1 µg/ml) for 20 min and washed and responses shown in panel B were elicited.

Time mark, 1 minute.

Fig. 14



In 2 experiments, after eliciting control responses at different frequencies, the preparation was restimulated in the presence of physostigmine (0.2 µg/ml) or hyoscine (0.1 µg/ml). Motor responses were potentiated by physostigmine (Fig. 15) at all frequencies, more so at lower frequencies. The motor responses at different frequencies were blocked by hyoscine. In one preparation from a 2 day old rabiit, higher frequencies (10 and 20 Hz) elicited relaxation after hyoscine treatment (Fig. 15).

4 day old rabbits

In preparations from 4 day old rabbits, responses to nerve stimulation were motor at 1 - 5 Hz and inhibitory at 10 and 20 Hz (Table 2). When the preparations were restimulated after exposure to NA, motor responses at 2 and 5 Hz were converted to relaxant responses, whereas the relaxant responses at 10 and 20 Hz were unaltered (Table 2).

6 day old rabbits

In 6 day old rabbit preparations, stimulation at lower

Fig. 15

Finleman preparation from 2 day old rabbit (suspended in McEwen's solution). Responses to periarterial nerve stimulation at different frequenices are indicated in Hz. Panel A shows control responses. Between panels A and B, the preparation was exposed to physostigmine (0.2 µg/ml) for 5 min and washed. Panel B shows responses to nerve stimulation in the presence of physostigmine. Between panels B and C, the preparation was exposed to hyoscine (0.1 µg/ml) for 5 min and washed. Panel C shows responses to nerve stimulation in the presence of hyoscine and to NA (200 ng/ml first response and 500 ng/ml second response). Time mark, 1 minute.

Fig. 15

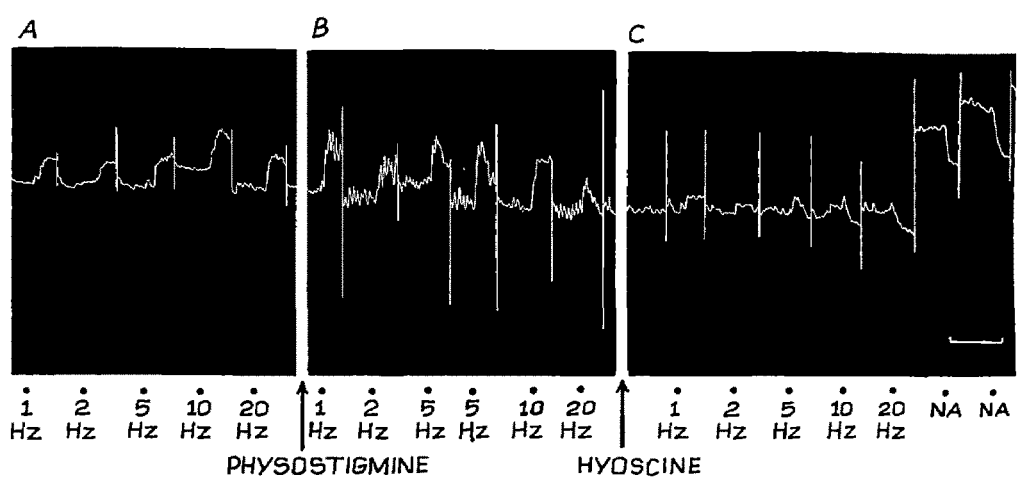
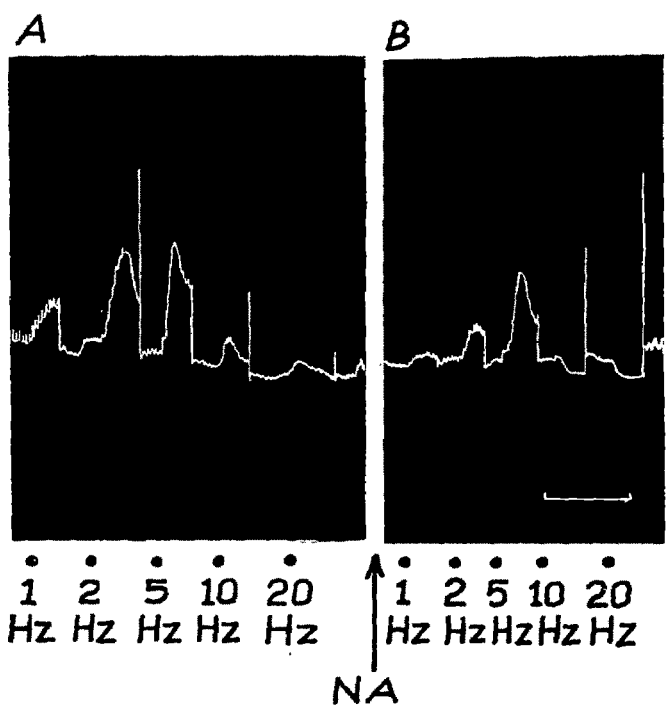

A
B
C
1 Hz
2 Hz
5 Hz
10 Hz
20 Hz
1 Hz
2 Hz
5 Hz
5 Hz
10 Hz
20 Hz
1 Hz
2 Hz
5 Hz
10 Hz
20 Hz
NA
NA
PHYSOSTIGMINE
HYOSCINE

Fig. 16

Finkleman preparation from 3 day old rabbit (suspended in McEwen's solution). Responses to periarterial nerve stimulation at different frequencies are indicated in Hz. Panel A shows control responses. Between panels A and B, the preparation was exposed to NA (1 µg/ml) for 20 min and washed and responses shown in panel B were elicited. Time mark, 1 minute.

Fig. 16



frequencies (1 and 2 Hz) elicited small motor response. Stimulation at higher frequencies (5, 10 and 20 Hz) elicited relaxant response. The contractile response at 1 Hz was abolished after exposure of the preparation to NA, whereas response at 2 Hz was reversed (Fig. 17). The relaxant responses at 5, 10 and 20 Hz were significantly ($P < 0.05$) potentiated after exposure to NA (Table 1; Fig. 17).

7 day old rabbits

In preparations from 7 day old rabbits, nerve stimulation elicited motor response at 1 Hz in one experiment, relaxant response in two experiments and no response in one experiment. At 2 Hz, there was no response in one experiment and relaxant response in three experiments. Stimulation at higher frequencies (5, 10 and 20 Hz) elicited relaxant responses in all the experiments (Table 2). The motor response at 1 Hz was abolished after exposure of the preparation to NA (1 µg/ml), whereas the inhibitory response at 2, 5, 10 and 20 Hz was either unaffected or potentiated (Table 2, Fig. 18).

Fig. 17

Finkleman preparation from 6 day old rabbit (suspended in McEwen's solution). Responses to periarterial nerve stimulation at different frequencies are indicated in Hz. Panel A shows control responses. Between panels A and B, the preparation was exposed to NA (1 µg/ml) for 20 min and washed and responses shown in panel B were elicited. Time mark, 1 minute.

Fig. 17

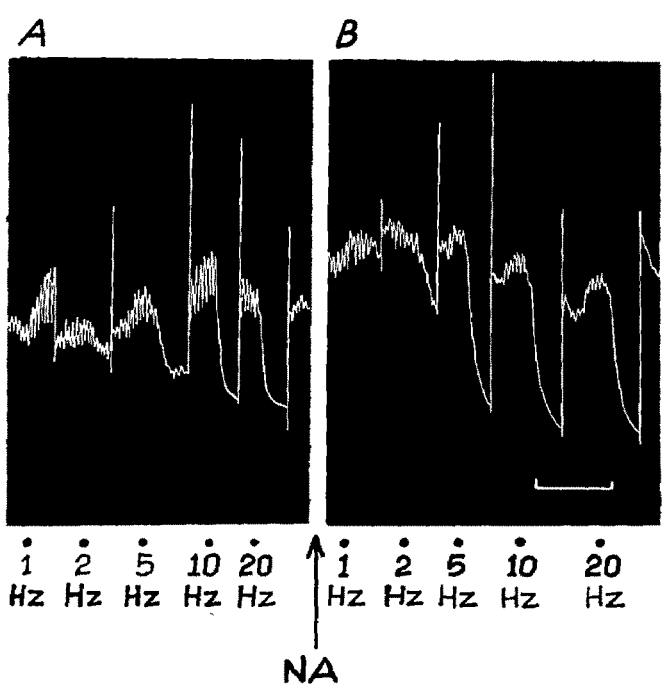
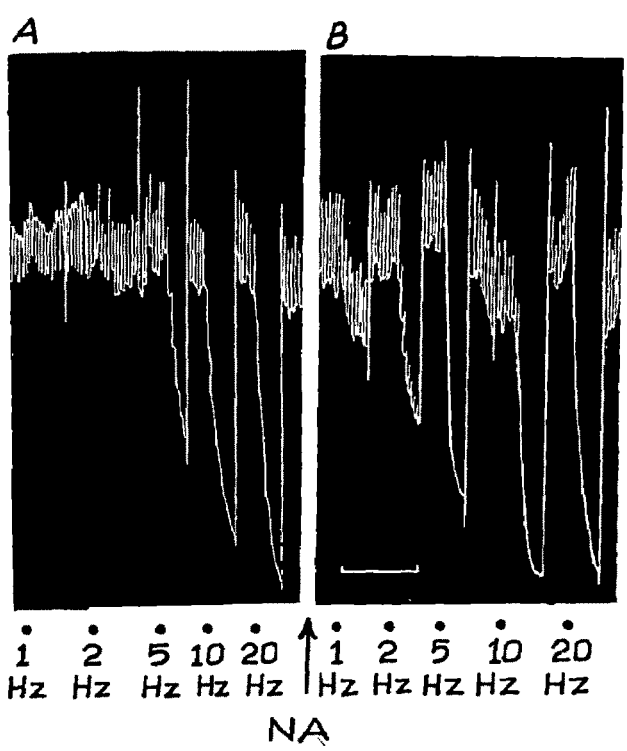


Fig. 18



10 day old rabbits

In preparations from 10 day old rabbits, stimulation at 1 and 2 Hz elicited motor response in two experiments and no response in two others. At 5, 10 and 20 Hz, only inhibitory responses were elicited. The motor responses at 1 and 2 Hz were converted to inhibitory responses after exposure of the preparations to NA (1 µg/ml), whereas the inhibitory responses at frequencies 5, 10 and 20 Hz were not affected (Table 2).

11 and 12 day old rabbits

In preparations from rabbits 11 and 12 day old, stimulation at all frequencies elicited inhibitory responses. After exposure to NA, the inhibitory responses were either potentiated or remained unaffected (Table 2, Fig. 19).

NA content of the ileum of rabbits of various age groups

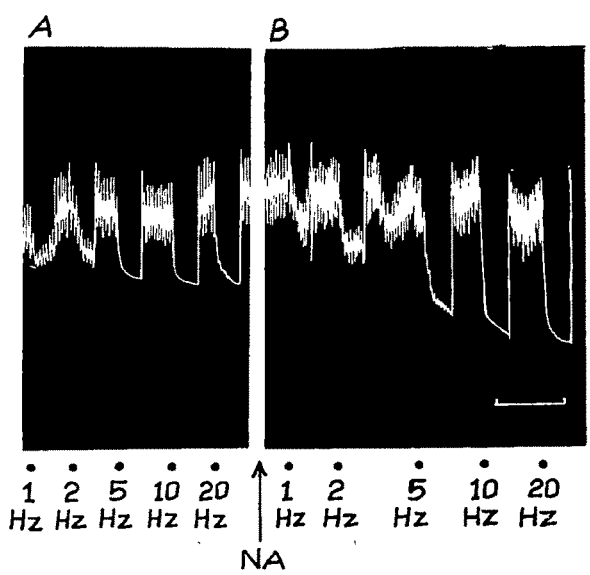
The data on the endogenous NA content of ileum of rabbits, 1 - 15 days old, are presentated in Table 3. The NA content was high at birth and remained so till the 6th day. There was

Fig. 19

Finkleman preparation from 12 day old rabbit (suspended in McEwen's solution). Responses to periarterial nerve stimulation at different frequencies are indicated in Hz. Panel A shows control responses. Between panels A and B, the preparation was exposed to NA (1 µg/ml) for 20 min and washed and responses shown in panel B were elicited.

Time mark, 1 minute.

Fig. 19



an abrupt decrease on the 7th day. A further gradual decrease occured over the next 5 days i.e. the 12th day of life. The contents on the 12th and 15th days of life were similar.

The NA content of ileum of adult rabbits were estimated in seven experiments. The value was 1.06 ± 0.04 µg/g.

Ability of ileum from rabbits 1 - 15 days to accumulate NA added exogenously

Ileum pieces from rabbits, 1 - 6 days old, accumulated 0.227 ± 0.028 to 1.085 ±0.021 µg/g of NA. This accumulation was cocaine-sensitive (Table 3). The ileum pieces from rabbits, 7 - 15 days old, accumulated, 2.85 ±0.021 to 3.30 ± 0.39 µg/g of NA. The accumulation increased with increase in the age of the animals (Table 3).

The data are plotted in Fig. 20 with the age of the animals on the abscissa and tissue (T) / medium (M) ratio of NA on the ordinate. The NA content of the medium incubating the ileum was not estimated but was derived by

TABLE - 3

Normal NA content of ileum of 1 - 15 day old rabbits and its ability to accumulate exogenously added NA.

Age	Normal NA content of ileum (µg/g±S.E.M.)	Control accumulation of NA* (µg/g ± S.E.M.)	Accumulation of NA in the presence of cocaine ** (µg/g ± S.E.M)	T/M ratio ± S.E.M.
1 day (6)	1.841 ± 0.16	0.227 ± 0.028	0.007 ± 0.008	0.227±0.03
2 day (5)	1.830 ± 0.04	0.542 ± 0.01	0.013 ± 0.027	0.543±0.02
3 day (4)	1.815 ± 0.06	0.698 ± 0.05	0.020 ± 0.018	0.703±0.01
6 day (4)	1.812 ± 0.10	1.085 ± 0.022	0.008 ± 0.03	1.088±0.03
7 day (4)	1.210 ± 0.33	2.850 ± 0.021	0.195 ± 0.11	2.965±0.4
9 day (4)	1.050 ± 0.032	3.120 ± 0.34	0.470 ± 0.23	3.182±0.36
12 day (4)	0.960 ± 0.05	3.260 ± 0.49	0.007 ± 0.05	3.400±0.5
15 day (4)	0.960 ± 0.06	3.30 ± 0.39	0.013 ± 0.03	3.450±0.18

* The tissue was exposed to NA (1 µg/ml) for 20 min at 35°C.

** The tissue was exposed to NA (1 µg/ml) and cocaine (3.5 µg/ml) for 20 min at 35°C.

For details of T/M (Tissue/Medium) ratio see text.

Figures in parentheses indicate the number of observations.

Fig. 20

Uptake of exogenously added NA (1 µg/ml) in vitro by ileum pieces of 1 - 15 day old rabbits. The age of the animals is on the abscissa, and tissue (T)/Medium (M) ratio of NA is on the ordinate. Close circle indicate T/M ratio obtained in the absence of cocaine and open circle indicate ratios in the presence of cocaine (3.4 µg/ml). Vertical lines represent S.E. of means.

Fig. 20

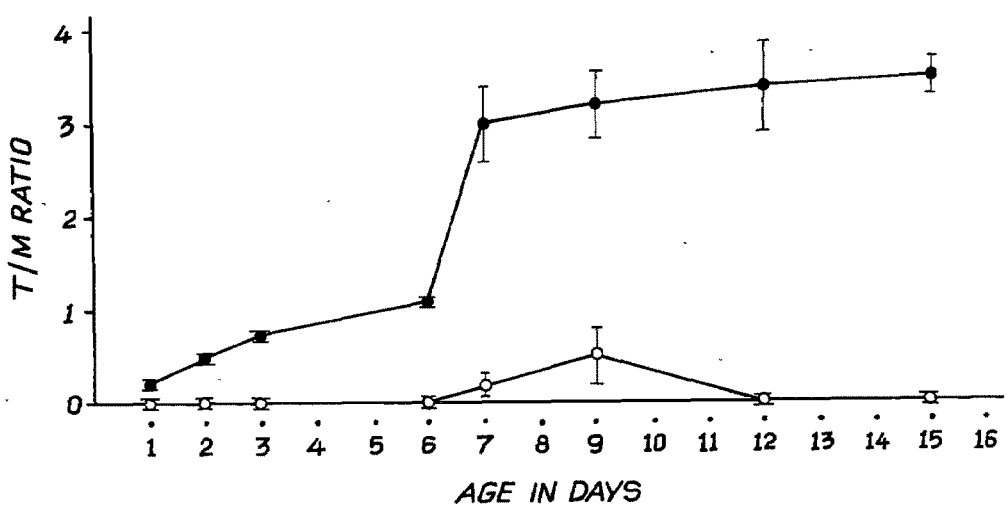


TABLE - 2

Effect of exogenously added NA on resposes to periarterial nerve stimulation of the isolated ileum preparations from young rabbits.

Age	Control response (mm ± S.E.M.)					Response (mm ± S.E.M.) after NA (1 µg/ml) for 20 minutes.				
	1 Hz	2 Hz	5 Hz	10 Hz	20 Hz	1 Hz	2 Hz	5 Hz	10 Hz	20 Hz
1 day (3)	3.6±0.3	4.7±2.1	6.0±1.5	11.0±2.07	12.0±0.3	0	0	0	0	0
2 day (3)	9.0±4.0	14.3±5.3	16.6±5.1	21.0±5.1	26.6±5.8	0	0	0	0	0
3 day (4)	5.3±1.8	6.6±0.4	9.6±1.5	14.3±0.9	17.0±4.3	0	0	0	0	0
4 day (3)	13.0±6.0	15.3±4.9	3.3±9.8	-11.6±1.7	-18.0±5.7	0	-5.0±0.0	-11.0±1.0	-16.7±2.1	-20.0±2.7
									P > 0.05	P > 0.05
6 day (4)	1.5±1.1	2.5±1.9	-8.0±1.4	-11.5±1.7	-12.8±0.7	0	-3.5±1.2	-13.5±0.6	-17.3±1.7	-18.0±1.8
								P < 0.05	P > 0.05	P < 0.05
7 day (4)	1.0±3.4	-5.0±2.8	-14.5±1.4	-33.0±3.4	-37.5±6.7	0	-9.7±3.9	-32.2±4.2	-43.2±5.9	-47.0±6.5
							P > 0.05	P < 0.05	P > 0.05	P > 0.05
10 day (4)	3.0±1.7	7.5±1.5	-19.0±10.4	-38.3±7.5	-43.5±8.9	-4.5±2.6	-7.5±4.4	-33.0±6.8	-48.5±6.3	-61.0±8.7
								P > 0.05	P > 0.05	P > 0.05
11 day(3)	-5.0±1.5	-7.0±2.0	-11.3±1.8	-28.6±3.2	-31.0±3.6	-8.1±1.1	-11.6±1.3	-16.0±1.0	-35.0±3.5	-40.0±1.5
						P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
12 day(3)	-3.0±3.0	-5.0±5.0	-46.0±11.8	-50.0±11.0	-53.0±11.2	-19.0±8.8	-23.8±6.5	-55.0±17.5	-64.0±13.5	-64.3±18.8
						P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05

Figures in parantheses indicate number of obervations

Negative sign indicates relaxation.

subtracting the amount accumulated in the tissue from the total amount (1 μg/ml, total 35 μg) present in the medium at the start of the experiment. It is clear from Fig. 20 that the T/M ratio increased with advancing age; the ratio was below 2 upto the 6th day of life and exceeded this value at the 7th day of life and beyond. The figure also demonstrates the effect of cocaine. In the presence of cocaine, the T/M ratio was less than unity at all ages.