Brit. J. Pharmacol. (1949), **4**, 323.

SOME RELATIONSHIPS BETWEEN ANTI-NICOTINE ACTIVITY AND SPECIFIC ANTAGONISMS

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(Received July 14, 1949)

Besides atropine, new synthetic compounds with parasympathicolytic and spasmolytic activity have been shown recently to possess therapeutic value in Parkinson's disease; these are diethylaminoethyl-1-phenyl-*cyclo*pentane-1-carboxylate (DPCPC) (Grünthal, 1946; Hartmann, 1946; Schwab and Leigh, 1949) and according to Sigwald *et al.* (1946) and Bovet *et al.* (1947, 1948) N-diethylaminoethylphenothiazine (DPT). The first is parasympathicolytic and papaverine-like (Domenjoz, 1946; Frommel *et al.*, 1949) and exhibits at the same time some antihistamine (Frommel *et al.*, 1949), peripheral curare-like (Domenjoz, 1946), and local anaesthetic (Fleisch and Baud, 1948) properties. In addition to these effects, Heymans *et al.* (1948a and b, 1949b and c) have demonstrated that DPCPC is a powerful anti-nicotine agent because it suppresses all the toxic effects of high doses of this alkaloid. The same authors have recently shown that DPT also exerts a strong anti-nicotine activity in protecting dogs against 100–200 lethal doses of nicotine (1949a, b, and c).

The very complex pharmacological activity of nicotine is generally divided into peripheral effects on muscular fibres and neuromuscular junctions, effects on autonomic ganglia, and effects on the central nervous system. Further and at each level, the action of nicotine depends on the alkaloid concentration and on the degree of intoxication, so that these can also be classified into motor and inhibitory effects (Bovet *et al.*, 1948; Goodman and Gilman, 1946). Corresponding to these modes and sites of action, nicotine can thus produce some effects on synaptic activity which, according to Langley and Dickinson (1889), can be used as a test of nicotine-like or anti-nicotine activity. On the other hand many other effects such as those on the respiratory, cardiovascular or central nervous system, or on isolated organs—e.g., intestine, vessels, or heart—do not show a clear relationship to the usual physiological stimulations. As it was supposed that the therapeutic activity of some compounds in Parkinson's disease might be related to their ganglionic-blocking effect on the tonic centres of the extrapyramidal tracts or on the cholinergic transmission (Sigwald *et al.*, 1946; Bovet *et al.*, 1947, 1948), we thought that it would be interesting to investigate more closely the relationship between anti-nicotine effect and parasympathicolytic or autonomic activity. For a better discrimination of such antagonisms, it seemed also necessary to extend our investigations to compounds of diverse chemical constitution possessing some specific activity such as antagonism of parasympathetic or sympathetic effects, spasmolysis, ganglionic-blocking, local anaesthesia, and even central depression.

We tested at first the overall antagonism against nicotine on the whole animal by determining protective indices.

METHOD

Mice weighing about 20 g. received intravenously a toxic dose of nicotine (0.00125 g./kg.) which produced convulsions, paralysis, and death in all animals. One hour previously, doses of the presumed protective agent were given subcutaneously to groups of 6–10 mice, and the ED50, protecting 50 per cent of animals, was determined according to Miller and Tainter's method (1944) which allows if necessary a statistical treatment of the results.

In a first series of experiments we tested the anti-nicotine effect of atropine as a true parasympathicolytic agent compared with DPCPC and DPT and of two synthetic compounds with neurogenic and myogenic activity (Meier, 1936; Johnson and Reynolds, 1937; Meier and Hoffmann, 1940; Graham and Lazarus, 1940; Tripod, 1949)—viz., "Trasentin" (diethylaminoethylester of diphenylacetic acid) and "Trasentin-H" (diethylaminoethylester of phenyl-*cyclo*hexylacetic acid). A specific sympathicolytic agent like ergotamine, and tetraethylammonium

bromide (TEA), which shows some anti-nicotine effect (Boelaert, 1948) besides its marked ganglionic-blocking action (Acheson *et al.*, 1945, 1946a and b; Trendelenburg, 1923), were also investigated. Procaine and cocaine were tested because the former possesses, if not a pure ganglion-blocking effect (Bovet *et al.*, 1948), at least some parasympathicolytic and anti-nicotine properties (Hazard *et al.*, 1942a and b; Moore *et al.*, 1948; Haimovici, 1948; Soehring and Hessler, 1949). Finally *d*-tubocurarine as a nerve-muscle blocking agent (Feldberg and Lin, 1949) and phenobarbitone as a general depressant of the central nervous system were used.

In a second series of determinations we tested in the same manner the anti-acetylcholine effect of all these compounds against an intravenous dose of acetylcholine (0.020 g./kg.) which produces convulsions, paralysis, and death in all animals.

Results

Table I gives the ED50 values for the anti-nicotine and anti-acetylcholine activities of all these agents.

TABLE I

ANTI-NICOTINE AND ANTI-ACETYLCHOLINE ACTIVITY OF VARIOUS AGENTS ON MICE

Class of drug	Drug	Anti-nicotine ED50 g./kg.	Anti-acetylcholine ED50 g./kg.
Parasympathicolytic and spasmolytic	Atropine	>0.800	0.009
	DPT	0.014	0.130
	DPCPC	0.031	0.013
	Trasentin	0.031	0.035
	Trasentin-H	0.085	0.018
Sympathicolytic	Ergotamine	>0.100	>0.100
Ganglion-blocking	Tetraethyl-ammonium	0.028	>0.200
Local anaesthetic	Cocaine	0.017	>0.200
	Procaine	0.100	>0.400
Peripheral-blocking	*d*-Tubocurarine	>0.00075	>0.00075
Central ...	Phenobarbitone	0.007	>0.100

These results show clearly that the general toxicity of nicotine can be antagonized by spasmolytic and ganglion-blocking agents or even by cocaine as well as by a central depressant. These findings seem thus to be related not only to a specific effect but also to various sites of action of the alkaloid. In addition, the lack of activity of atropine is very striking so that a parasympathomimetic effect of nicotine is unlikely. Further, this test also clearly demonstrates that a good anti-nicotine activity is not exclusively exhibited by DPT and DPCPC, since trasentin and to some extent trasentin-H can also antagonize the effects of nicotine.

On the other hand, an anti-acetylcholine activity is shown only by atropine and by spasmolytic agents which are known to possess parasympathicolytic properties like DPT (Bovet *et al.*, 1947, 1948; Gordon, 1948; Heymans *et al.*, 1949a, b, and c), DPCPC (Domenjoz, 1946; Bovet *et al*, 1948; Heymans *et al.*, 1948a and b; Frommel *et al.*, 1949), trasentin, and trasentin-H (Meier, 1936; Meier and Hoffmann, 1940; Graham and Lazarus, 1940; Bovet *et al.*, 1948; Tripod, 1949).

TABLE II

RELATIONSHIP BETWEEN ANTI-NICOTINE AND ANTI-ACETYLCHOLINE ACTIVITIES

Drug	Index $\frac{\text{anti-nicotine potency}}{\text{anti-acetylcholine potency}}$
Atropine	<0.01
DPT	9.30
DPCPC	0.42
Trasentin	1.13
Trasentin-H ...	0.21

The ratios between the ED50 values for the anti-nicotine and anti-acetylcholine activities (Table II) demonstrate that these properties are clearly independent in these spasmolytic compounds.

Since an anti-nicotine activity on the whole animal is exhibited by various compounds, which are neither chemically nor pharmacologically related, it seemed necessary to compare the effect of nicotine with typical autonomic effects in a simpler test.

Acetylcholine has a consistently stimulant effect on isolated rabbit and guinea-pig ileum. Nicotine on the contrary produces on the intestine a contraction as described by Trendelenburg (1917), Alvarez (1918a and b), von Oettingen *et al.* (1928), and recently by Feldberg and Lin (1949), or an inhibition of the activity with higher concentrations according to Langley and Magnus (1905). This effect can be mixed or biphasic (Alvarez, 1918), and depends on the dose, the anatomical localization of the intestinal strip, the animal species, as well as on the experimental technique (Alvarez, 1918; Raymond-Hamet, 1930; Bovet

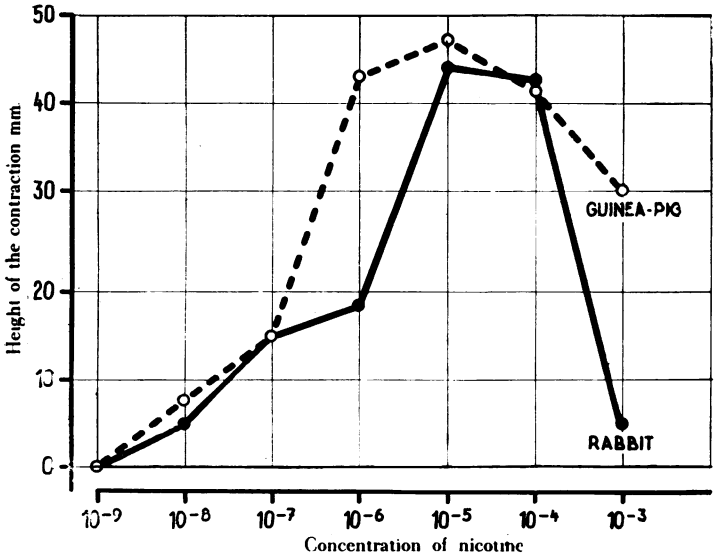


FIG. 1.—Relationship between concentration of nicotine and height of contraction of the isolated guinea-pig and rabbit ileum.

et al., 1948). It seemed therefore necessary to establish a test in which a typical motor effect of nicotine would be produced.

METHOD

Isolated strips of rabbit or guinea-pig ileum were kept at 0° during four hours and afterwards suspended in a 50 c.c. bath containing oxygenated Tyrode. The bath temperature was 38° C. and a frontal writing lever was used. The concentrations mentioned in the text are the final concentrations in the bath.

RESULTS

The mean response-dose curves for nicotine on the rabbit and guinea-pig ileum are depicted in Fig. 1.

Guinea-pig ileum seemed suitable for the testing of a nicotine antagonism because its motor effect has a wider range; moreover, a biphasic response is only elicited by concentrations higher than 10^{-4}, and the contractions can be reproduced quite regularly without tachyphylaxis, at least with low concentrations as already described by Feldberg and Lin (1949). Rabbit ileum, on the other hand, reacts more irregularly to the alkaloid and shows even in low concentrations, for example 10^{-7}, a biphasic or polyphasic effect.

The antagonistic activities of various agents were tested on isolated guinea-pig ileum against a final nicotine concentration of 10^{-6}. The anti-nicotine effects recorded after the prophylactic treatment with the antagonist could then be measured and the inhibition statistically treated for "all or none" (Morrell *et al.*, 1940; Miller *et al.*, 1948) or graded responses (Schild, 1942; Holton, 1948; Tripod, 1949). Figs. 2 and 3 illustrate the antagonistic effects of atropine and trasentin-H. The values of the concentrations producing a 50 per cent anti-nicotine effect (ED50)

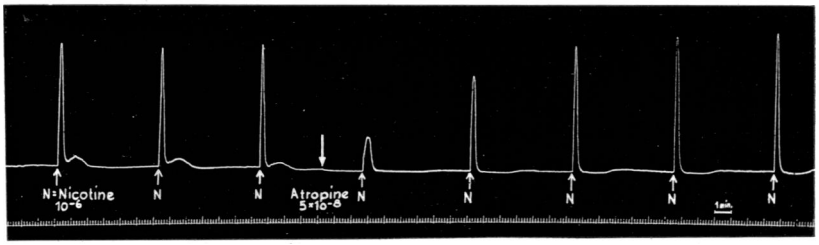
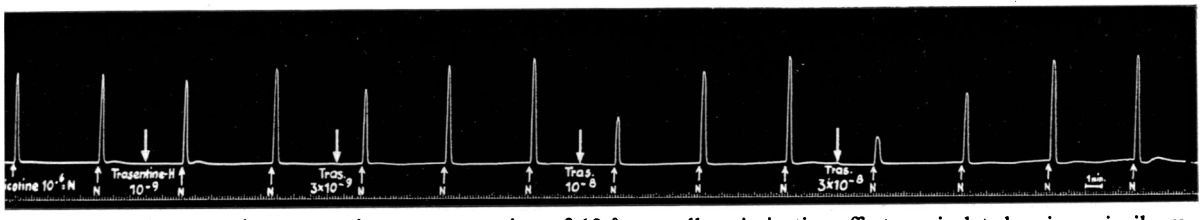


FIG. 2.—The anti-nicotine effect of 5×10^{-8} atropine is illustrated on isolated guinea-pig ileum.



G. 3.—Trasentin-H produces even in a concentration of 10^{-9} a small anti-nicotine effect on isolated guinea-pig ileum. The inhibition of the motor effect of nicotine is marked with 3×10^{-8}.

TABLE III

ANTI-NICOTINE ACTIVITY OF VARIOUS AGENTS ON ISOLATED GUINEA-PIG ILEUM

Class of drug	Drug	Anti-nicotine ED50	Relative potency
Parasympathicolytic and spasmolytic	Atropine	2×10^{-8}	100
	DPT	1×10^{-7}	20
	DPCPC	2×10^{-8}	100
	Trasentin	6×10^{-8}	33
	Trasentin-H	2×10^{-8}	100
Sympathicolytic	Ergotamine	10^{-5}	0.2
	Priscol	10^{-5}	0.2
Ganglion-blocking	Tetraethyl-ammonium	$>10^{-4}$	<0.02
Local anaesthetic	Cocaine	$>10^{-4}$	<0.02
	Procaine	10^{-5}	0.2
Peripheral-blocking	*d*-Tubocurarine	3×10^{-4}	0.006
Central ...	Barbitone	$>10^{-4}$	<0.02
	Phenobarbitone	10^{-4}	0.02

have been graphically evaluated from several experiments and are listed in Table III, where the relative potency of atropine is put as 100.

The greatest anti-nicotine activity is strikingly shown by atropine and the spasmolytic compounds, whereas sympathicolytic, ganglion-blocking, local anaesthetic, and peripheral-blocking agents as well as central depressants all show an antagonism, but in such high concentrations that their specificity is rather doubtful. In their recent paper Feldberg and Lin (1949) drew attention to the anti-nicotine effect of local anaesthetics and of *d*-tubocurarine on the guinea-pig and the rabbit ileum; in our experiments on guinea-pig gut, however, this inhibition was found to be much smaller than that of atropine.

In the same manner we tested on the rabbit ileum the antagonism of some agents against a final concentration of acetylcholine of 5×10^{-7}. The ED50 values are compiled in Table IV, where the relative potency of atropine is again 100.

As expected, only atropine and atropine-like agents are here effective, but the spasmolytic compounds possess different potencies for anti-nicotine and anti-acetylcholine activity as shown by Tables III and IV, although both are produced by rather similar concentrations.

Further, we compared various antagonistic effects, in order to establish the relationships between the anti-nicotine specificity, the sympathicolytic activity against 3×10^{-6} adrenaline on the seminal vesicle of the guinea-pig (Brügger, 1945), and the antagonism against 2×10^{-4} $BaCl_2$ on the rabbit ileum.

TABLE IV

ANTI-ACETYLCHOLINE ACTIVITY OF VARIOUS AGENTS ON ISOLATED RABBIT ILEUM

Class of drug	Drug	Anti-acetylcholine ED50	Relative potency
Parasympathicolytic and spasmolytic	Atropine	2.3×10^{-8}	100
	DPT	4×10^{-7}	5.7
	DPCPC	2×10^{-7}	11.5
	Trasentin	4.4×10^{-7}	5.2
	Trasentin-H	1.6×10^{-7}	14.4
Ganglion-blocking	Tetraethyl-ammonium	3.2×10^{-4}	0.007
Local anaesthetic	Cocaine	2.3×10^{-4}	0.01
	Procaine	2.1×10^{-5}	0.11
Peripheral-blocking	*d*-Tubocurarine	$>10^{-4}$	<0.02
Central ...	Phenobarbitone	$>10^{-4}$	<0.02

TABLE V

RELATIONSHIP BETWEEN ANTI-NICOTINE EFFECT AND OTHER ANTAGONISMS ON ISOLATED ORGANS

Drug	Indices of specific potencies		
	Anti-nicotine / Anti-acetylcholine	Anti-nicotine / Sympathicolytic	Anti-nicotine / Musculotropic
Atropine ...	1.1	3,000	50,000
DPT ...	4	30	100
DPCPC ...	10	1,000	500
Trasentin	7.3	333	167
Trasentin-H	8	1,000	150

Calculated ratios of anti-nicotine activities to other antagonistic activities are reproduced in Table V. They show that the anti-nicotine activity is more closely related to the anti-acetylcholine activity than to any of the other antagonisms.

On the isolated guinea-pig ileum it can thus easily be demonstrated that atropine and spasmolytic compounds like DPT, DPCPC, trasentin, and trasentin-H exert a specific antagonism against the nicotinic stimulation, antagonism which is not related to anti-adrenaline or musculotropic properties. A closer relationship is only exhibited with

an anti-acetylcholine effect, so that this point seemed worthy of further investigation. Therefore, we tested the antagonistic effect of these agents against other substances with muscarine-like properties such as arecoline and pilocarpine (Bovet *et al.*, 1948) and determined the various antagonistic ED50 values on the guinea-pig ileum against acetylcholine (10^{-7}), arecoline (3×10^{-8}), and pilocarpine (5×10^{-7}) (Tables VI and VII).

TABLE VI

ANTAGONISMS ON ISOLATED GUINEA-PIG ILEUM

Drug	Anti-acetyl-choline ED50	Anti-nico-tine ED50	Anti-areco-line ED50	Anti-pilo-carpine ED50
Atropine ...	7×10^{-9}	2×10^{-8}	7×10^{-10}	2×10^{-9}
DPT ...	2×10^{-7}	10^{-7}	2×10^{-8}	4×10^{-8}
DPCPC ...	8×10^{-8}	2×10^{-8}	2×10^{-8}	3×10^{-8}
Trasentin ...	4×10^{-7}	6×10^{-8}	3×10^{-8}	6×10^{-8}
Trasentin-H	2×10^{-8}	2×10^{-8}	2×10^{-9}	3×10^{-9}

TABLE VII

RELATIONSHIP BETWEEN ANTI-NICOTINE EFFECT AND OTHER ANTAGONISMS ON ISOLATED GUINEA-PIG ILEUM

Drug	Indices of specific potencies		
	Anti-nicotine / Anti-acetylcholine	Anti-nicotine / Anti-arecoline	Anti-nicotine / Anti-pilocarpine
Atropine ...	0.35	0.035	0.1
DPT ...	2.0	0.20	0.4
DPCPC ...	4.0	1.00	1.5
Trasentin ...	6.7	0.50	1.0
Trasentin-H	1.0	0.10	0.15

Similar experiments showed that tetraethylammonium, cocaine and procaine, as well as *d*-tubocurarine, exhibit only an unspecific antagonism against these muscarine-like substances in concentrations higher than 10^{-5}.

Discussion

These findings show that anti-acetylcholine, anti-nicotine, anti-arecoline, and anti-pilocarpine properties are closely related but that, nevertheless, differences of specificity occur. In spite of this, it seems that the group of spasmolytics investigated all exhibit the same type of antagonism against a muscarine-like effect.

The site of action of nicotine on the isolated guinea-pig ileum has thus to be considered. Its motor effect could theoretically be due to a ganglionic excitation by low concentrations of the alkaloid. In our experiments, however, it was demonstrated that a specific ganglionic-blocking compound like tetraethylammonium cannot antagonize the stimulation produced by 10^{-6} nicotine (Table III). As our experiments were done on ice-treated ileum in order to obtain a greater and more regular sensitivity to nicotine, it might be objected that this treatment already "blocks" the autonomic ganglia of the intestine; this is not the case because we obtained the same values as those listed in Table III with the ileum of freshly killed guinea-pigs, with perhaps a little higher sensitivity to cocaine, procaine, and *d*-tubocurarine which, however, still remains 100–1,600 times smaller than for atropine. A usual ganglionic site of action being excluded, the motor effect of nicotine can be explained by special pharmacological properties of the intestinal ganglia of the guinea-pig, or by a peripheral action on the neural elements—e.g., a post-ganglionic stimulation. The analogy with arecoline and pilocarpine which also have a postganglionic and parasympathomimetic action (Bovet *et al.*, 1948) strengthens this hypothesis. Moreover, a post-ganglionic action of nicotine has already been demonstrated on the blood vessels by Haimovici (1948).

In any case, an anti-nicotine activity on the whole animal seems to be related to various mechanisms involved in the different sites of action of nicotine. This effect is not exclusively a property of spasmolytic compounds with therapeutic activity in Parkinson's disease like DPT and DPCPC, since trasentin and trasentin-H equally show a good anti-nicotine effect, and atropine does not show it at all. Furthermore, the anti-nicotine activity on isolated organs is comparable to an anti-acetylcholine or anti-muscarine mechanism, and it is again exhibited with high specificity not exclusively by spasmolytic compounds with therapeutic activity in Parkinson's disease. It seems thus that the therapeutic activity of DPT and DPCPC is produced by a different and characteristic mechanism, but neither the anti-acetylcholine nor the anti-nicotine mechanism shows a convincing parallelism with therapeutic activity in Parkinson's disease.

Summary

1. On mice, the toxicity of nicotine can be antagonized by spasmolytic and ganglionic-blocking agents as well as by a central depressant. These anti-nicotine properties seem to be related to various sites of action of the alkaloid and not to a specific blocking of parasympathetic effects.

2. On the isolated guinea-pig ileum nicotine produces a muscarine-like stimulation which can be antagonized by atropine and spasmolytic compounds. These anti-nicotine properties are related more to an anti-acetylcholine or anti-muscarine effect than to a sympathicolytic or musculotropic activity.

We are grateful to Mr. Brüni and Mr. Bubendorf for their technical assistance in these experiments.

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