THE ACTION ON NICOTINE-INDUCED TREMORS OF SUBSTANCES EFFECTIVE IN PARKINSONISM

D. BOVET AND V. G. LONGO

Laboratorio di Chimica Terapeutica dell'Istituto Superiore di Sanita, Rome, Italy

Received for publication December 20, 1950

In 1946 clinical observations made independently in France by Sigwald *et al.* and in Switzerland by Grünthal attested the therapeutic activity of two new products, Diparcol[1] and caramiphen[2] (Panparnit), against Parkinson's disease; this activity was later confirmed by many authors. With respect to their pharmacodynamic action, these preparations cannot be classified as a single, discrete pharmacological group. They exhibit spasmolytic, ganglionic blocking and parasympatholytic properties (Domenjoz, 1946; Bovet *et al.*, 1947; Heymans and de Vleeschhouver, 1948) which in no way suggest their clinical applicability for the relief of symptoms caused by diseases of the basal ganglia. Drugs which have been introduced to relieve the spasticity, tremor and other symptoms of extrapyramidal disease have, in the past, been evaluated almost entirely by clinical observations. There is, at present, no laboratory method for the screening and assaying of drugs potentially effective in various disorders in which rigidity and tremors are characteristic.

The present investigation stems from our observation that Diparcol and Panparnit exert an action antagonistic to the convulsive effects elicited by intravenous injections of nicotine. Animals previously treated with small doses of these drugs scarcely react to nicotine injection and the convulsions produced by nicotine in non-treated animals can be abolished completely by adequate doses. It is well known that nicotine, in addition to affecting ganglia and the cardiovascular system in mammals, stimulates motor centers. Furthermore, when applied locally on the rolandic area, nicotine causes a substantial lowering of the threshold of excitation and sometimes produces uncoordinated movements, clonus and convulsions (Amantea, 1920). An attempt was made, therefore, to discover whether or not a correlation could be found between the suppression of nicotine-induced tremors in animals and clinical effectiveness in Parkinsonism.

Recently, clinical reports have indicated beneficial results in Parkinsonism after treatment with Artane (Corbin, 1949), some antihistaminic drugs, diphenhydramine (Benadryl) and Phenergan (Gair and Ducey, 1950), and with miscellaneous drugs such as amphetamine and mephenesin (Myanesin). These drugs, together with atropine, hyoscine, Diparcol, Panparnit and other agents similar to them in structure and pharmacologic properties, were tested to find a possible parallelism between well-defined pharmacological characteristics and clinical activity in Parkinsonism.

[1] Diethylamino-ethyl-N-phenothiazine hydrochloride.

[2] Diethylaminoethyl ester of phenylcyclopentane carboxylic acid hydrochloride.

METHOD. The method consists in the observation of the capacity of a drug to counteract the tremors elicited by the intravenous injection of nicotine. Although this antagonism is evident in other species, rabbits were used exclusively in these experiments.

The intravenous injection (marginal ear vein) of 1 mgm./kgm. of nicotine bitartrate (1:500 aqueous solution) causes, in 80 per cent of rabbits, a symptom complex of salivation, dyspnea and characteristic tremors of the limbs. Uncoordinated movements of the limbs generally cause the animal to fall on its side. For more precise investigation a graphic recording of the tremors of a posterior limb can be obtained (see fig. 1).

The agents to be tested were administered intravenously five minutes before treatment with nicotine. The experiments were carried out with groups of four rabbits (average weight: 2 kgm.), with a corresponding control group that received only nicotine; the results were expressed in fractions corresponding to the number of animals that showed convulsions over the total number that were injected.

RESULTS. *Effect of drugs active in Parkinson's disease.* These data are summarized in table 1. There is a rather satisfactory parallelism between laboratory and clinical results.

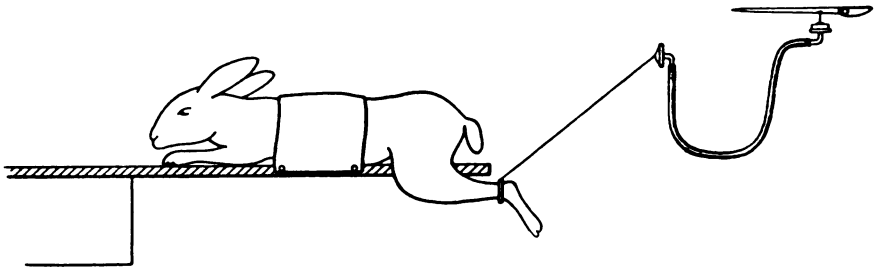


FIG. 1. Method of graphic registration of the muscular twitchings of a rabbit's leg. The animal is secured on a wooden table. The hind legs hang free, the front legs are fixed to the table. The twitchings of the leg are recorded by means of a Marey's tambour for kymographic recording.

Panparnit, Diparcol and Artane counteracted efficaciously the tremors produced by nicotine. Each of these agents was effective in a dose of 2 mgm./kgm. In a dose of 5 mgm./kgm. Panparnit completely suppressed the tremors while Diparcol and Artane in a similar dose did not produce complete suppression (fig. 2).

Six antihistaminic drugs were tested in a similar manner. Of the six, clinical reports of more or less marked activity in Parkinsonism have been made for all save chlorcyclizine. The drugs tested in this group can be classified in the following order of decreasing nicotinolytic power: Phenergan, Benadryl, chlorcyclizine (Perazil), dimenhydrinate (Dramamine), pyranisamine (Neo-Antergan), phenindamine (Thephorin). The weak activity of Neo-Antergan in this test may be ascribed to its highly specific antihistaminic action and its recognized lack of central effects.

As examples of spasmolytic drugs, adiphenine (Trasentine), Pavatrine and 92 G.T.[3] were tested; both Trasentine and 92 G.T. proved very active, while Pavatrine was inactive in the doses tested.

[3] β-diethylaminoethyl-2-phenyl-2-(hydrocyclopentyl)ethanoate hydrochloride.

TABLE 1

Drug actions against tremors elicited by nicotine (1 mgm./kgm. i.v.)

DRUG	CONTROLS	TREATED			
	No. convulsed / No. injected	Minutes after treatment	mgm./kgm.		
			2	5	10
Diparcol	16/20	5	2/4	1/4	0/4
		30	2/4	1/4	1/4
		60	3/4	2/4	1/4
Panparnit	17/20	5	2/4	0/4	0/4
		30	2/4	0/4	0/4
		60	3/4	2/4	1/4
Artane	16/20	5	2/4	1/4	1/4
		30	2/4	1/4	1/4
Benadryl	8/10	5		1/4	Toxic
		30		2/4	
		60		4/4	
Phenergan	8/10	5		1/4	0/4
		30		2/4	0/4
		60		2/4	2/4
Dramamine	8/10	5			1/4
		30			3/4
Neo-Antergan	8/10	5		2/4	1/4
		30			2/4
Thephorin	8/10	5		2/4	2/4
Perazil	8/10	5			1/4
		30			1/4
Trasentine	17/20	5	2/4	0/4	0/4
		30	2/4	0/4	0/4
		60	3/4	2/4	1/4
92 G.T.	17/20	5	1/4	0/4	0/4
		30	2/4	0/4	0/4
Pavatrine	4/4	5	4/4	Toxic	
Myanesin	8/10	5		4/4	4/4
Amphetamine	8/10	5		2/4	2/4
		30		2/4	2/4
Atropine	8/10	5		4/4	4/4
Hyoscine	8/10	5		4/4	4/4

Amphetamine, although of a completely different chemical series, showed some suppressive action against the tremors elicited by nicotine, but did not modify its effects on the neurovegetative system.

Myanesin, atropine and hyoscine proved to be inactive. The negative results with Myanesin can be related to the distinctive mechanism of action of this drug (Henneman, Kaplan and Unna, 1949; Henneman and Scherrer, 1949). The same reasoning applies to atropine and hyoscine.

Effect of other agents. From a pharmacological point of view it became of interest to investigate whether or not this antagonism to nicotine-induced tremors was common to various types of drugs. In this regard it was shown above that atropine, a classic parasympatholytic preparation, did not antagonize the nicotinic tremors.

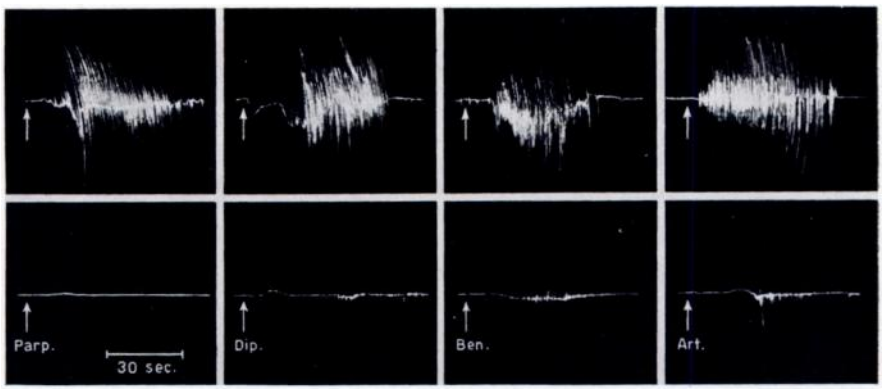


FIG. 2. Graphs of muscular twitchings of a rabbit's leg. Each graph is from a separate animal. Top: at arrows, nicotine bitartrate (1 mgm./kgm. i. v.). Bottom: same dose of nicotine administered five minutes after treatment with various drugs used for Parkinson's disease (5 mgm./kgm. i. v.). Parp. = Panparnit; Dip. = Diparcol; Ben. = Benadryl; Art. = Artane.

Since Diparcol and Panparnit had been shown to exert ganglionic blocking actions, it was possible that this action might be responsible for their antinicotine properties. Under the same experimental conditions, however, tetraethylammonium bromide (TEA), pentamethylenebistrimethylammonium iodide (C5), procaine and sparteine did not show any action against the tremors caused by nicotine. By means of a technique previously described (Longo, 1950), it was possible to show, however, that TEA, C5, Diparcol and Panparnit all antagonized the cardiac slowing produced by nicotine (table 2). Thus a distinction could be made between the blocking action at ganglia and at motor centers.

Dibenamine, phenobarbital, hexobarbital (Evipal) and paraldehyde proved to be inactive against the nicotinic tremors; the last two did weaken the tremors but only at hypnotic dose levels (table 3). This lack of antagonism does not agree with findings recently reported by Tripod (1949) who found that these nonspecific drugs reduced nicotine toxicity in mice.

Effect of Diparcol and Panparnit on other types of tremors. In order to deter-

mine whether the action against nicotine was dependent upon a general anticonvulsant property as other authors reported (Heymans and de Vleeschhouver, 1948; Beck *et al.*, 1949), Diparcol and Panparnit were tested at the same dosage

TABLE 2

Antagonism against central and peripheral effects of nicotine in the rabbit

	ACTION AGAINST TREMORS ELICITED BY NICOTINE (1 mgm./kgm. i.v.)	ACTION AGAINST HEART-SLOWING ELICITED BY NICOTINE (0.4 mgm./kgm. i.v.)
	Active dose	Active dose
	mgm./kgm.	*mgm./kgm.*
Diparcol	10	10
Panparnit	10	10
TEA	75*	20
C5	Inactive	2
Sparteine	Inactive	5

* Very transitory effect.

TABLE 3

Action of various drugs against tremors elicited by nicotine (1 mgm./kgm. i.v.)

	CONTROLS	TREATED				
	No. convulsed / No. injected	Minutes after injection	mgm./kgm. i.v.			
			5	10	50	100
Morphine sulfate	8/10	5	3/4	4/4		
Paraldehyde*	8/10	5		4/4	3/4	0/4
Phenobarbital	9/10	5	4/4	4/4		
Evipal†	8/10	5	4/4	0/4		
Dibenamine	8/10	5	4/4	4/4		

* Sol. 2% in isotonic glucose.

† Evipal sodium 1%.

TABLE 4

CONVULSANT	CONTROLS	AFTER TREATMENT WITH:	
	No. convulsed / No. injected	Diparcol 10 mgm./kgm. i.v.	Panparnit 10 mgm./kgm. i.v.
Strychnine, 0.2 mgm./kgm. i.v.	3/4	3/4	2/4
Metrazol, 25 mgm./kgm. i.v.	4/4	0/4*	3/4†

* The tremors were only partially abolished.

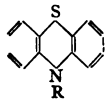
† Extensor tonic component of seizure abolished.

against other convulsant drugs, strychnine and pentylenetetrazole (Metrazol). The results obtained are summarized in table 4. Neither Panparnit nor Diparcol displayed any antagonistic action against the convulsions produced by strychnine; Diparcol appeared to have some effect against the tremors caused by Metrazol, while Panparnit showed a negligible weakening power.

Relationship between anti-nicotine action and therapeutic results in a series of products derived from phenothiazine. A series of phenothiazine derivatives, including Diparcol and Phenergan, have been tested in clinics. A systematic laboratory comparison was performed to study the mechanism of their action and to evaluate the method. The tests were made with the following preparations: 1) Dimethylamino-N-phenothiazine-hydrochloride (3015 R.P.); 2) Diethylaminoethyl-N-phenothiazine-hydrochloride (Diparcol); 3) (Dimethylaminoethyl-2'-methyl-2')-ethyl-phenothiazine-hydrochloride (Phenergan); 4) (Diethylamino-

TABLE 5

Action of various phenothiazine derivatives against tremors elicited by nicotine (1 mgm./kgm. i.v.)

	CONTROLS	TREATED			
	No. convulsed / No. injected	Minutes after injection	mgm./kgm.		
			2	3	10
3356 R.P. $R=CH_2-CH(CH_3)-N(C_2H_5)_2$	17/20	5	1/4	0/4	
		30	2/4	0/4	
		60	3/4	0/4	
Diparcol $R=CH_2-CH_2-N(C_2H_5)_2$	26/30	5	2/4	1/4	0/4
		30	2/4	1/4	0/4
		60	2/4	2/4	1/4
Phenergan $R=CH_2-CH(CH_3)-N(CH_3)_2$	27/30	5	2/4	0/4	0/4
		30	2/4	1/4	0/4
		60			0/4
3015 R.P. $R=CH_2-CH_2-N(CH_3)_2$	8/10	5	4/4	4/4	4/4
		30			
3580 R.P. $R=CH_2-CH_2-N(C_2H_5)_3$	9/10	5	4/4	4/4	Toxic at 6 mgm.

2'-methyl-2') ethyl-N-phenothiazine-hydrochloride (3356 R.P.); 5) Diethylaminoethyl-N-phenothiazine-ethyl-iodide (3580 R.P.).

Durel (1949) and Sigwald (1949) showed that the average active doses of 3356 R.P. and Phenergan were clinically equivalent but that the percentage of improvement was somewhat higher with the first product. Good results were obtained with Diparcol in higher doses. The drug 3015 R.P., even in large doses, had little effect; the drug 3580 R.P. had practically no effect. The laboratory trials against the tremors caused by nicotine corresponded perfectly with the clinical tests showing a good antagonistic action for 3356 R.P., for Phenergan and for Diparcol, and negligible actions for 3015 and 3580 (tables 5 and 6).

Discussion. The mechanism of action of drugs acting in Parkinson's disease is not yet completely clear. Domenjoz (1946) has described the curarizing action

of Panparnit on frogs, and Fleisch and Baud (1948) concluded that the same drug had an action on proprioceptive receptors; however, opposed to these observations are the experiments of Heymans and de Vleeschhouver (1948) who found no effect on the indirect excitation of muscle (chloralosed dog), and those of Gruber *et al.* (1949) who found a facilitating action on muscle. That the mechanism of action of the agents of this group is central, as postulated by Sigwald *et al.* (1946) for Diparcol and by Szatmary (1948) for Panparnit, has been confirmed experimentally. In fact, Gruber *et al.* (1949) observed that in the cat Panparnit eliminates decerebrate rigidity; similar results were obtained using Trasentine and amprotropine (Syntropan) while no relaxation was noted after large doses of atropine and hyoscine.

Churchill and Gammon (1949) noted an antagonistic action of Benadryl to the clinical and electroencephalographic signs of "petit mal". The authors concluded that this action was central, and precisely, that it was on the reticular system of the thalamus which has been proved by electroencephalography to be the origin of these discharges of "petit mal".

TABLE 6

			3356 R.P.	DIPAR-COL	PHE-NERGAN	3015 R.P.	3580 R.P.
Laboratory trials	Action against tremors elicited by nicotine		++++	+++	+++	0	0
Clinical trials	Average dose active mgm./day p. o.		250	750	200	400	1000
	No. patients treated		106	342	38		
	% improved	Hypertony	87	77	82		
		Tremors	86	71	70	Small activity	No activity

It appears that our results on the antagonism of the various "anti-Parkinson" drugs against nicotine can be interpreted in the same way. The present observations confirm the classic data on the appearance of nicotinic convulsions of central origin and show that they are not analogous to those caused by strychnine and Metrazol: The tremors caused by nicotine are mild and brief, are not preceded by a tonic phase and are associated with more important neurovegetative symptoms; furthermore, the pharmacological antagonisms are different in each case. Indeed, while the drugs which antagonize Metrazol convulsions correspond well enough to the group of anti-epileptic drugs (of which phenobarbital is the most typical example), the antagonistic effects toward nicotine generally appear to be characteristic of the so-called "anti-Parkinson" preparations.

From these data the following observations stand out: 1) preparations active in the treatment of Parkinson's disease possess anti-nicotine actions; 2) there is no parallelism between the anticonvulsant effect against nicotine and against other excitants of the central nervous system; 3) in an homologous series, the drugs which have a strong antagonism toward the central effects of nicotine are the ones which are effective against Parkinson's disease.

Nicotine, of course, can stimulate at the synapses in sympathetic and parasympathetic ganglia as well as stimulate the central nervous system. Not all anti-nicotine drugs have actions at all these sites. Particularly important in this respect are the actions of TEA and C5, drugs which antagonize the effects of nicotine on ganglia but do not antagonize the nicotinic convulsions of central origin (Longo, 1950).

These observations are considered to be a new argument in favor of the development of a central action by cholinergic preparations. It is possible to propose that the antagonism observed at the ganglia between acetylcholine and nicotine-like products on the one hand, and anti-nicotine preparations on the other, exists also at the central level. It does not seem unreasonable, on this basis, to think that the drugs effective against Parkinson's disease block, at the level of the mesencephalic and bulbo-pontine centers, the effects of a cholinergic transmission no longer harmonically controlled by the superior center, destroyed or deeply injured by disease.

SUMMARY

1. It has been found that there is a parallelism between the antagonism of some drugs (Diparcol, Panparnit, Artane) against the tremors produced by nicotine, and the satisfactory clinical results obtained with these drugs in the symptomatic therapy of Parkinson's disease.

2. Other drugs, such as Benadryl, Phenergan, Trasentine and amphetamine show the same central antagonistic effect; they are also effective in the clinical treatment of Parkinsonism. This effect seems not to be connected with antihistaminic, spasmolytic, sympathomimetic or anesthetic properties.

3. The comparison between five preparations of similar chemical composition derived from dibenzoparathiazine has afforded proof of a complete similarity between the antagonism that the drugs show to the central effects of nicotine and their usefulness in the treatment of Parkinson's disease.

4. Tetraethylammonium bromide and pentamethylenebistrimethylammonium iodide, which antagonize the peripheral action of nicotine, do not antagonize the tremors induced by nicotine.

5. The results are discussed with respect to the mechanism of action of drugs useful in the treatment of Parkinson's disease.

Acknowledgment: We wish to thank Prof. R. Paul and Dr. P. Viaud (Rhone-Poulenc, Paris) for the products R.P. (Diparcol, Phenergan and others); Dr. R. Domenjoz (Geigy, Basle) for the Panparnit; Dr. R. O. Roblin (American Cyanamid Co., Stamford, Conn.) for the Artane; and Dr. G. Treves (Schieffelin and Co., New York) for the 92 G.T.

REFERENCES

AMANTEA, A.: Arch. Farm. Sper., **30:** 3, 1920.

BECK, I. T., FROMMEL, E., FAVRE, M., AND VALLETTE, F.: Arch. Internat. de Pharmacodyn. et de Therap., **78:** 613, 1949.

BOVET, D., FOURNEL, J., AND CHARPENTIER, P.: Therapie, **2:** 115, 1947.

CHURCHILL, J. A., AND GAMMON, G. D.: J.A.M.A., **141:** 18, 1949.

CORBIN, K. B.: J.A.M.A., **141:** 377, 1949.
DOMENJOZ, R.: Schweiz. Med. Wchnschr., **50:** 1282, 1946.
DUREL, P.: J. de Medecine de Lyon, **706:** 431, 1949.
FLEISCH, A., AND BAUD, C.: Helv. Physiol. et Pharmacol. Acta, **6:** 331, 1948.
GAIR, D. S., AND DUCEY, J.: Arch. Int. Med., **85:** 284, 1950.
GRUBER, C. M., KRAATZ, C. P., GRUBER, C. M., JR., AND COPELAND, J. E.: THIS JOURNAL, **96:** 11, 1949.
GRÜNTHAL, E.: Schweiz. Med. Wchnschr., **50:** 1286, 1946.
HENNEMAN, E., KAPLAN, A., AND UNNA, K.: THIS JOURNAL, **97:** 331, 1949.
HENNEMAN, E., AND SCHERRER, J.: THIS JOURNAL, **97:** 342, 1949.
HEYMANS, C., AND DE VLEESCHHOUVER, G. R.: Arch. Internat. de Pharmacodyn. et de Therap., **75:** 307, 1948.
LONGO, V. G.: Boll. Soc. Ital. Biol. Sper., **26:** 564, 1950.
SIGWALD, J.: Therapie, **4:** 205, 1949.
SIGWALD, J., BOVET, D., AND DUMONT, G.: Revue Neurologique, **78:** 11, 1946.
SZATMÁRY, A., AND MESZAROS, A.: Monthly Rev. Psychiat. and Neurol., **116:** 2, 1948.
TRIPOD, J.: Brit. J. Pharmacol., **4:** 323, 1949.