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Effect of Some Centrally Acting Drugs on Compulsive Circling. (22034)

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The group of signs and symptoms observed, following a unilateral intracarotid injection of di-isopropyl fluorophosphate (DFP) into rabbits, has been called the adversive syndrome (1) and the latter has been attributed to the anticholinesterase action of DFP(1-5). As a result of DFP administration, an asymmetric distribution of cholinesterase (ChE) activity is produced in the frontal cortex and caudate nucleus. The enzymatic activity is profoundly depressed on the injected side of the brain whereas the opposite side is relatively unaffected. This is especially marked in the right caudate nucleus which retains only 4% of its normal ChE activity. This inhibition permits asymmetric accumulation of acetylcholine (ACh) in the brain(6) which in turn may be directly responsible for the observed effects. It has been established in cats and monkeys that the adversive syndrome is the result of a biochemical lesion of the central nervous system(7). Although it is possible that peripheral receptors are more important in rabbits than in cats(7), many factors also point to a central mechanism for the production of the adversive syndrome in the former animal. First, the direct application of DFP to the peripheral vestibular receptors elicits no behavioral change in rabbits(8). Secondly, central stimulants can modify the forced circling behavior(5). Thirdly, a characteristic asymmetric brain cholinesterase pattern is found in all animals exhibiting the adversive syndrome, and this pattern is absent when compulsive movements are not observed (3,9,10). Finally a temporary or permanent reversal in the direction of the forced movements usually follows a minor convulsive seizure(2). The factors mentioned above appear to implicate the central nervous system as the primary site for the production of compulsive circling in rabbits. Previous work(5) showed that the adversive syndrome can be corrected by intravenous injections of small amounts of atropine sulfate after which the animal exhibits its normal mode of progression. When large doses (i.v.) of atropine are given the forced circling is reestablished, however, the animal turns in the opposite direction, toward the injected side.

In the present study a further analysis of

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TABLE I. Effect of Atropine-Like Drugs on the Adversive Syndrome (Dose of Drug, mg/kg).

Drug	Reversal after atropine*			Reversal without atropine		
	No. of exp.	Mean	Range	No. of exp.	Mean	Range
Scopolamine	5	.04	.02 -.08	4	.09	.06 -.12
Artane	8	.05	.02 -.10	6	.07	.02 -.12
Panparnit	6	.2	.1 -.3	5	.2	.1 -.4
WIN 2299	5	.015	.005-.02	5	.013	.005-.025

* Adversive syndrome was corrected by intravenous administration of atropine before experimental drug was injected.

this reversal is based on the ability of a variety of centrally acting drugs to produce this phenomenon. It is worth noting that the preparation to be described may be used as a bioassay for the atropine-like properties of drugs which act on the central nervous system. This bioassay is valuable because it establishes the drug action in terms of a behavioral response, namely the reversal in direction of the compulsive circling, in contrast to other assays which measure the drug effect on a single isolated organ or tissue.

Method. The adversive syndrome was produced in albino rabbits (approximately 2 kg) as described previously(5). The following drugs were used: atropine sulfate, scopolamine (hyoscine hydrobromide), Artane (trihexyphenidyl), Panparnit (diethyl aminoethyl-1-phenylcyclopentane-1-carboxylate hydrochloride), WIN 2299 (2-diethyl aminoethyl cyclopentyl-(2-thienyl) glycolate hydrochloride), Benadryl hydrochloride (diphenydramine hydrochloride), Diparcol (diethylaminoethyl-N-dibenzoparathiazine hydrochloride), LSD (d-Lysergic acid diethylamide), Reserpine (an alkaloid from Rauwolfia serpentina Benth), and Lergigan [N (2-dimethyl amino-2 methylethyl) phenothiazine]. All drug injections were made into a marginal ear vein. The *average values* (mg/kg) for a single intravenous injection of each drug were: atropine 0.2, scopolamine 0.02, Artane 0.02, Panparnit 0.1, WIN 2299 0.005, Benadryl 0.2, Diparcol 1.0, LSD 0.01, Reserpine 0.5, and Lergigan 0.2. Following each intravenous injection of a test drug, the rabbit was allowed to move freely on a rubber mat. The injections were continued at 2 to 6 minute intervals until the direction of the forced circling was reversed from that seen immediately after the administration of DFP. This reversal of forced circling was used as the end point in establishing the effect of the drug on the CNS. In those cases where no reversal of the forced movements was noted the injections were continued until the animals appeared to be in respiratory difficulty, in convulsions, or asleep. The last effect was noted only after Reserpine. In some cases atropine was administered prior to the test drug. This was done to obtain information on the synergism of atropine and the test drugs.

Results. The data obtained with atropine-like drugs are shown in Table I. In these experiments the amount of drug necessary to produce a reversal of the adversive syndrome was established in 2 groups of animals: 1) following atropine correction, and 2) without prior atropine administration.

The direction of the forced circling was reversed by a mean dose of 0.04 mg/kg of scopolamine when atropine was first injected in amounts sufficient to correct forced circling. However, 0.09 mg/kg of this drug was needed without atropine. The difference in these means was significant to beyond the 1% level when tested by the Student "t" test. Therefore, the pharmacological effects of scopolamine and atropine appear to be additive in our preparation.

The other 3 atropine-like drugs included in Table I, Artane, Panparnit and WIN 2299, required approximately the same dose of drug to produce a reversal in the direction of the forced circling whether or not atropine was first administered. In contrast to the results obtained with scopolamine, a synergism was not noted when these 3 drugs were used with atropine.

The effect on the adversive syndrome of some other drugs known to act on the CNS are presented in Table II. In general, these

TABLE II. Effect on Adversive Syndrome of Some Drugs Known to Act on the CNS (Dose of Drug, mg/kg).

Drug	No reversal			Reversal		
	No. of exp.	Mean	Range	No. of exp.	Mean	Range
Lergigan	3	2.7	1.2 - 3.5	5	1.8	1.2-2.8
Benadryl	8	3.0	1.0 - 6.6	2	.6	
Reserpine	5	4.6	3.0 - 6.0			
Diparcol	3	9.2	4.5 -12.0	1	5.0	
LSD	5	.05	.015- .09	1	.02	

drugs do not have a strong atropine-like action and were therefore administered to the rabbits without prior atropine treatment. Of the five drugs tested, Lergigan was best able to reverse the direction of the forced circling. It should be noted that a reversal was observed in 5 of the 8 animals studied.

Benadryl, a potent anti-histaminic reversed the direction of forced circling in only 2 of 10 animals tested. It was therefore far inferior to Lergigan in its ability to change the direction of forced circling. *Reserpine* does not affect the forced circling. Each of the 5 rabbits given this drug appeared to fall asleep with a scoliosis away from the injected side. A gentle tapping on the spine aroused the animal and it then circled about several times before resuming its sleeping posture. *Diparcol* reversed the involuntary movements in only one of the 4 rabbits tested. However, the mean dose needed was much larger than that of the other drugs listed in Table II. *LSD* was similar to Diparcol in its lack of a marked effect on forced circling. The direction of the compulsive movement was reversed in only one of the animals studied. In general, it appeared to be quite toxic when administered following the intracarotid injection of DFP.

Additional observations were made on ability of the listed drugs in Table II to correct the adversive syndrome. The corrected animal exhibits a normal behavioral pattern and may move in any direction. In a given animal the correction always occurs with a smaller dose of drug than that needed for reversal. In the present experiments this phenomenon was observed only with Lergigan and Benadryl. In the case of Benadryl 4 of the 10 rabbits were corrected, whereas 7 of 8 other rabbits studied were corrected by Lergigan.

Discussion. It has been previously reported (5) that small amounts of atropine correct the forced circling produced in rabbits by the unilateral intracarotid injection of DFP. This effect was referred to the power of atropine to block the action of accumulated ACh. On the other hand, large doses of atropine restored forced circling, but in the opposite direction. Since several CNS stimulants can also evoke a reversal in forced circling, it was suggested that this latter effect of atropine was due to a stimulant action. The fact that stimulation of the cortex may be important in producing a reversal is given further support from the observation that a mild convulsive seizure may be followed by such a change in the direction of the forced movements.

Of the drugs used in our study, the reversal in forced circling was most easily obtained with the atropine-like ones. However, it is apparent that the syndrome can be produced by a variety of drugs. These may be roughly divided into two categories: a) the CNS stimulants and b) the atropine-like drugs.

The suprabulbar vestibular system is complicated and the mechanism by which it is affected in the present preparation is unknown. However, two possible hypotheses may be suggested to explain the production of the reversed adversive syndrome: (a) the more nearly normal enzyme content of the left cortex may allow the intravenously injected drug selectively to stimulate that side more than the enzymatically depressed right side, thereby resulting in a reversal in the direction of forced circling, or (b) the production of convulsive activity on both sides of the cortex is followed by a bilateral depression in cortical activity. Both of these mechanisms would "neutralize" the original asymmetric activity of the cortex which follows the unilateral intracarotid injection of DFP. In the first case

(a) the reversal is then attributed to a stimulation of the left cortex by the injected drug and in the second (b) to the biochemical lesion in the right caudate nucleus which appears to be so severe as to preclude restoration of normal function by the administration of atropine or other drugs. It has been shown (11) that ablation of the right caudate nucleus (with or without destruction of the cerebral cortex) results in circling toward the right. The brain areas involved in the production of the adversive syndrome as well as the mechanisms by which drugs affect this system are at present the subject of a series of investigations in our laboratory.

The usefulness of the present method as a bioassay is demonstrated by the results obtained with the experimental drug WIN 2299. It is shown to be similar to Artane, Panparnit and Lergigan in its effect on forced circling. These drugs play an important role in the treatment of Parkinsonism. Clinical trials with WIN 2299 would seem to be profitable from our present results.

Summary. The intravenous injection of scopolamine, Artane, Panparnit, Lergigan or WIN 2299 into rabbits exhibiting the adversive syndrome evoke a reversal in the direction of the compulsive circling. Of the drugs mentioned, only scopolamine acts synergistically with atropine in causing this reversal. The usefulness of the procedure outlined as a bioassay for atropine-like drugs is discussed and two possible mechanisms by which the drugs studied produce this reversal are mentioned.

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Practicability of Shipping HeLa Cell Suspensions for Use in Color Test For Poliomyelitis Antibodies.* (22035)

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The continuous cultivation *in vitro* of the human cancer cell (HeLa) by Gey and coworkers(1), and the important finding that these cells showed cytopathic effects in the presence of poliomyelitis virus(2), has made possible the use of many tests for poliomyelitis investigations without maintaining a monkey colony. Small laboratories are now better able to make such investigations providing ready access to a good supply of HeLa cells is available. The authors have previously reported their observations on the quantitative titration of 2000 human sera against 3 prototype strains of poliomyelitis virus employing as indicator system color changes of medium in tubes containing suspended HeLa cells(3).

This report concerns the practicability of employing suspensions of HeLa cells shipped to a laboratory for use in carrying out the same method.† The observations were undertaken in order to: (a) learn whether a small

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