Brit. J. Pharmacol. (1955), **10**, 383.

THE PHARMACOLOGICAL PROPERTIES OF THE OPTICAL ISOMERS OF BENZHEXOL, PROCYCLIDINE, TRICYCLAMOL AND RELATED COMPOUNDS

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(RECEIVED MAY 25, 1955)

The powerful peripheral atropine-like ("anti-muscarinic") actions of benzhexol, procyclidine, and its quaternary derivative tricyclamol, are described by Cunningham, Harned, Clark, Cosgrove, Daugherty, Hine, Vessey, and Yuda (1949), Montuschi, Phillips, Prescott, and Green (1952) and Lee, Gibson, Dinwiddie, and Mills (1954) respectively. The atropine-like properties of related quaternary amino*cyclo*hexyl-carbinols have also been mentioned (see Adamson, Barrett, and Wilkinson, 1951). Benzhexol and procyclidine are used for symptomatic relief in Parkinson's disease and tricyclamol is used for its spasmolytic action on smooth muscle.

Only the racemic forms of the above compounds have so far been described. We have separated the optical isomers and have shown that the anti-muscarinic action is largely confined to the *laevo* isomers, most of which are not more toxic than the corresponding *dextro* isomers, and do not differ greatly from them in certain other respects.

METHODS

Chemical

Resolution was effected by crystallization of the acid D tartrates from ethyl alcohol, successive crops of solid being removed until only oily material would separate. Ether was added as necessary to facilitate crystallization. The solid consisted chiefly of *dextro* base D tartrate with the *laevo* base D tartrate remaining in the oil and mother liquor. Each was made alkaline with ammonia and the base crystallized from ethanol. The first crops were always of pure optically active base, later crops being of the racemic form.

The hydrochlorides crystallized from ethanol-ethyl acetate mixture.

The quaternary salts were obtained by addition of methyl or ethyl iodide to a solution of the appropriate base in acetone. They were recrystallized from acetone.

Pharmacological

The two optical isomers and the racemic mixtures of each compound were simultaneously compared with atropine by the mydriatic test in mice (Ing, Dawes, and Wajda, 1945), and by their activities in relaxing ACh-induced spasm of isolated guinea-pig ileum. In the mydriatic tests activity was estimated from the pupil diameters 30 min. after intraperitoneal injection of two or three doses of each compound, using groups of ten mice. The ileum tests were carried out in the manner previously described (Green, 1953) but using ACh (5×10^{-7}) to induce spasm. LD50's were determined by intravenous injection in groups of white Swiss mice, using a 2:3 dose ratio.

Vagal bradycardia or contraction of the nictitating membrane was elicited in cats under chloralose anaesthesia by submaximal stimulation of the cut vagus or cervical sympathetic nerves drawn into fluid electrodes. The stimulus consisted of D.C. pulses at 20 cycles/sec. from a blocking oscillator (Dickinson, 1950), applied for periods of 3 or 5 sec.

The peristaltic reflex of isolated guinea-pig ileum was recorded by Trendelenburg's method and elicited by raising the internal pressure to 2.5 cm. water.

Pain thresholds in the analgesic tests were determined by the pressure method of Green and Young (1951).

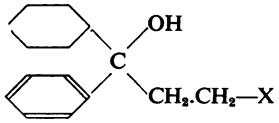
RESULTS

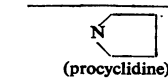
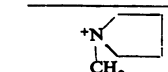
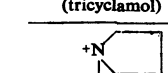
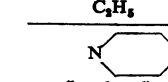
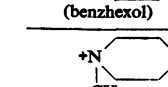
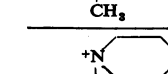
The activities of the *laevo* isomers, tested by mydriasis in mice or relaxation of ACh-induced spasm of guinea-pig ileum, were about twice those of the *racemic* mixtures, and far greater than those of the respective *dextro* isomers, except for the ethiodide of procyclidine, where the differences in activity were relatively small, but in the same direction; optical rotations suggested incomplete separation of the isomers (Table I). In contrast, the *laevo* isomers, except of the ethiodide of benzhexol, were no more toxic than the *dextro* isomers, and those of procyclidine and tricyclamol were significantly less toxic ($P<0.001$).

TABLE I

COMPARISON OF (1) PERIPHERAL ATROPINE-LIKE ACTIVITIES (ESTIMATED BY MYDRIASIS IN MICE OR RELAXATION OF ACETYLCHOLINE SPASM OF GUINEA-PIG ILEUM) AND (2) INTRAVENOUS TOXICITY (IN MICE)

General formula:



X	Isomer	m.p., °C.	Rotation $[\alpha]_{5461}^{20}$ C=0·4 in $CHCl_3$	Relative Activity Atropine Sulphate=1·0		LD50† mg./kg.
				Mydriasis	Guinea-pig Ileum	
 (procyclidine)	*Racemate*	227		0·035	0·05	79
	Dextro	246	+30°	<0·003	0·002	69
	Laevo	246	−30°	0·060	0·10	86
 (tricyclamol)	*Racemate*	204–5		0·35	0·66	13 (a)
	Dextro	180	+25°	0·01	0·01	9 (a)
	Laevo	180	−25°	0·62	1·6	14 (a)
	*Racemate*	157–8		0·40	0·4	14 (a)
	Dextro	165	+30°	0·004	0·0034	12 (a)
	Laevo	165	−30°	0·76	1·0	14 (a)
 (benzhexol)	*Racemate*	241–3		0·064	0·28	60
	Dextro	264	+31°	0·025	0·075	54
	Laevo	264	−30°	0·12	0·71	57
	*Racemate*	202–3		0·46	0·57*	15*
	Dextro	183–4	+23°	0·034	0·018	12
	Laevo	183–4	−22°	1·1	0·86	14
	*Racemate*	179–180		0·32		14
	Dextro	175	+21°	0·11	Not tested	17
	Laevo	175	−18°	0·41		11
Atropine sulphate				1·0	1·0	90

* Not tested on the same occasion as the isomers. † Groups of 10 mice were used except for those estimates marked (a) − 20 mice per group.

The limits of error of the estimates of relative potency, for P=0·95, are of the order of ±20% in the mydriatic tests and ±35% in the tests on guinea-pig ileum.

Further comparison of the isomers of procyclidine and tricyclamol is presented below.

Isomers of Procyclidine

Peripheral Atropine-like Effects.—The *laevo* isomer possesses greater activity than the *dextro* by the methods referred to in Table I, and in other tests. In relaxing ACh-induced spasm of rabbit ileum, the potencies of the racemate, *dextro* isomer and *laevo* isomer were respectively 0.08, 0.0015, and 0.15 times that of atropine sulphate. In cats, under pentobarbitone sodium, the depressor effect of small doses of ACh was temporarily reduced by as little as 0.01 mg./kg. of the *laevo* isomer, and almost eliminated by 0.1 mg./kg., whereas the *dextro* isomer at doses of 2 to 5 mg./kg. only slightly reduced the effect of ACh. Vagal bradycardia was similarly affected.

Compared by their parasympatholytic effect (pressor action) in a cat infused with carbachol and adrenaline (method of Bülbring and Dawes, 1945), the activities of the racemate, *dextro* and *laevo* isomers were, respectively, approximately 0.06, 0.002, and 0.13 times that of atropine sulphate.

For protection against lethal doses of eserine (1 mg./kg. i.v.) the minimal effective doses determined in groups of ten mice were for the racemate, *dextro* isomer and *laevo* isomer, 1.5, 50, and 0.8 mg./kg. respectively.

Toxicity.—The *laevo* isomer was less toxic than the *dextro* isomer by the intravenous route in mice (Table I). Rabbits were more sensitive to both isomers; the lethal intravenous dose of each in this species was 6–10 mg./kg., and with either isomer cardiac failure preceded respiratory arrest; both isomers caused tremors and convulsions. In cats under pentobarbitone sodium, rapid falls in blood pressure (30–60 mm. Hg) occurred after 2 to 4 mg./kg. of either isomer, recovery being complete

in 2–3 min. Larger doses (10 mg./kg.) caused respiratory arrest and were tolerated only if artificial respiration was given.

Other Effects.—The isomers caused about equal inhibition of the pendular movements of the longitudinal muscle of strips of isolated rabbit ileum. In a comparative test, by adding the drugs in random order to 25 ml. baths, at the rate of 0.1 mg./min. from a mechanically driven syringe, the pendular movements of the ileum were reduced by 50% (mean of 4 trials) by the racemate at 1.2×10^{-5}, the *dextro* isomer at 1.2×10^{-5} and by the *laevo* isomer at 1.05×10^{-5}.

Little antihistamine action was shown. Thus, in relaxing histamine-induced spasm of guinea-pig ileum the activities of the racemate, *dextro* and *laevo* isomers were respectively 0.003, 0.0023, and 0.0049 times that of mepyramine. Contractions of the nictitating membrane due to pre- or postganglionic stimulation were not affected by intravenous injection of 5 mg./kg. of either isomer. Neither isomer caused analgesia or reduced the action of morphine (4 mg./kg.) when given subcutaneously at doses of 50 mg./kg. and neither at 12.5 mg./kg. intravenously reduced the duration of anaesthesia with 75 mg./kg. pentobarbitone sodium intravenously. Oxytocic action was not shown by either isomer at 10^{-6} to 10^{-5}, and the same concentrations did not prevent adrenaline (2×10^{-7}) from causing maximal contraction of isolated rabbit uterus.

Isomers of Tricyclamol

Peripheral Atropine-like Effects.—The greater anti-muscarinic action of the *laevo* isomer is shown in Table I and in other tests. Thus, in relaxing ACh-induced spasm of isolated rabbit ileum the activities of the *dextro* and *laevo* isomers were approximately 0.006 and 2 times that of atropine sulphate respectively. Further, in inhibiting the stretch reflex of isolated guinea-pig ileum the *laevo* isomer (effective at about 10^{-8}) was much more active than the *dextro* (effective at 10^{-6} to 3×10^{-6}) and about twice as potent as the racemate. Again, in cats under pentobarbitone, electrically induced vagal bradycardia was abolished and the threshold to intravenous ACh was increased by over tenfold after 0.1 mg./kg. of the *laevo* isomer, whereas more than 3 mg./kg. of the *dextro* isomer was necessary to cause similar effects. After large doses of the *laevo* isomer (2 mg./kg.) the nicotinic pressor action of ACh (500 μg.) could be observed.

Toxicity.—Respiratory arrest and paralysis of the sciatic nerve-gastrocnemius muscle preparation occurred with each isomer at doses of about 20 mg./kg. in the anaesthetized cat. Higher doses were tolerated under artificial respiration. In conscious rabbits, or in those anaesthetized with urethane, respiratory failure was again the main toxic action, occurring with either isomer or the racemic mixture at doses of 10–20 mg./kg. In mice the *dextro* isomer was slightly less toxic than the *laevo* isomer (Table I).

Other Effects.—Unlike the analogous tertiary amine (procyclidine), tricyclamol blocks the superior cervical ganglion. Like the neuromuscular effect, this action is exhibited to approximately the same extent by the *laevo* and *dextro* isomers, 5 mg./kg. intravenously causing about a 70% reduction in the contractions of the nictitating membrane due to preganglionic stimulation, without affecting those due to simultaneous postganglionic stimulation of the opposite membrane. These doses lowered the blood pressure, this action tending to be slightly greater with the *dextro* than with the *laevo* isomer.

Neither analgesia nor reduction in the analgesic effect of morphine sulphate (4 mg./kg. subcutaneously) occurred after subcutaneous injection of 50 mg./kg. of either isomer in rats, and the duration of anaesthesia due to 75 mg./kg. pentobarbitone sodium in mice was not affected by either isomer. They do not show any specific antihistamine action (guinea-pig ileum and cat blood pressure), oxytocic effect (isolated guinea-pig or rabbit uterus) or anti-adrenaline action (isolated rabbit uterus or cat blood pressure). At high concentrations (3×10^{-6}–10^{-5}) both reduced the spasmogenic action of $BaCl_2$ (5×10^{-5}) on isolated guinea-pig ileum.

Summary

1. The separation of the optical isomers of procyclidine, benzhexol, and their respective methiodides and ethiodides is described.

2. The anti-muscarinic activity of these compounds is almost entirely due to the *laevo* components.

3. The anti-muscarinic action is not related to the toxicity of the compounds, most of the *laevo* isomers being no more toxic and some (e.g. procyclidine and tricyclamol) being slightly less toxic than the *dextro* isomers.

4. In other respects the effects of the *dextro* and *laevo* isomers of the procyclidine and tricyclamol do not differ greatly ; for example, the *dextro* and *laevo* isomers of tricyclamol possess equal, though feeble, ganglion-blocking and neuromuscular-blocking actions.

The authors are indebted to Dr. D. W. Adamson and Dr. A. C. White for their interest in this work, and to Mrs. E. P. Penson and Mrs. I. A. Saunders, who carried out many of the pharmacological tests.

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