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ACTIONS OF HALOPERIDOL, MEPERIDINE, AND RELATED COMPOUNDS ON THE EXCITABILITY AND ION CONTENT OF ISOLATED CEREBRAL TISSUE

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Abstract—Haloperidol, two other γ-aminobutyrophenones structurally related to it, meperidine and chlorpromazine were compared in their actions on isolated tissues from guinea pig cerebral cortex. Under ordinary metabolic conditions, the compounds were without effect on the respiration and on the sodium and potassium content of the tissues, and also on their chloride content and glycolysis in the instances examined. Response of the tissues to electrical stimulation was, however, highly sensitive to the compounds, all of which inhibited respiratory response at a concentration of 10 μM. At higher concentrations, haloperidol increased the sodium content of the stimulated tissue. Chlorpromazine also acted in this fashion, but the two other γ-aminobutyrophenones and meperidine, did not do so.

HALOPERIDOL (I, Fig. 1) has characteristic actions on excitability and ion movements in electrically stimulated cerebral tissues in isolation, which have been reported briefly[1] and are now described in more detail. The systematic development of haloperidol from meperidine[2–4] involved selecting compounds for specified types of central

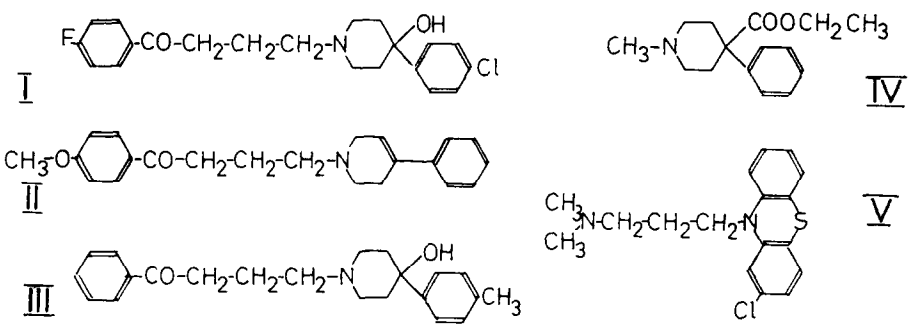

FIG. 1. Compounds examined: I, haloperidol; II, anisoperidone; III, R1636; IV, meperidine; V, chlorpromazine.

activity, and in successive groups of compounds those selected became chlorpromazine-like rather than analgesic. Electrically-stimulated cerebral tissues are sensitive also to chlorpromazine,[5] and the biologically less active compounds from which haloperidol was selected, were therefore examined to see if they shared the actions found in haloperidol or chlorpromazine. For this purpose, Dr. P. A. J. Janssen

kindly made available to us the additional substances shown in Fig. 1, and their behaviour with isolated cerebral tissue is now reported, together with that of meperidine.

METHODS

Tissue metabolism. Guinea pigs were stunned by a blow at the back of the neck, exsanguinated, the brain removed, and slices of cerebral cortex 0·35 mm thick cut with a blade and glass guide. The "recommended procedure" of McIlwain and Rodnight[6] was followed except when tissue was cut without added fluid, when method (ii) of Bachelard *et al.*[7] was used. Six slices from the two hemispheres were successively weighed, floated to fluid media (at 37° in the case of method (ii)), mounted in electrodes, and the electrodes placed in their vessels, which already contained oxygenated incubation fluid. This point was reached approx. 15 min after stunning the animal.

Manometric experiments employed glycylglycine-buffered fluids equilibrated with O_2 at 37°; CO_2 was absorbed by NaOH-soaked paper in a centre-well and readings of O_2 pressure taken each 5 min. Other experiments employed quick-transfer apparatus[6] and were carried out with bicarbonate-buffered saline in equilibrium with 5% CO_2–95% O_2 at 37°.

Incubation fluids. Normal media were based on Krebs–Ringer solutions and were of the following composition (mM). Bicarbonate medium: NaCl, 120; KCl, 4·75; KH_2PO_4, 1·2; $MgSO_4$, 1·2; $CaCl_2$, 2·6; $NaHCO_3$, 25, and glucose, 10; inulin, when specified, 1%. Glycylglycine medium: NaCl, 127; KCl, 5·1; KH_2PO_4, 1·3; $MgCl_2$, 1·3; $CaCl_2$, 2·7; glycylglycine, 30; brought to pH 7·4 by NaOH, which contributed about 4 mM Na.

Substances whose metabolic effects were being examined were present in the incubation fluid in the concentrations quoted, in 3–6 of the 6–8 vessels of typical experiments, before tissues were added. Haloperidol and related compounds were taken to solution in an excess of tartaric acid, subsequently neutralized (P. A. J. Janssen, personal communication); 1 mM tartaric acid, added in control experiments, was without effect on the characteristics measured.

Extraction and analysis. Most metabolic experiments were terminated by rapidly releasing the tissues successively, to a dish containing 20–30 ml ice-cold 0·32 M sucrose. From this each was lifted within 2 sec with a mounted, bent wire and dropped to 4 ml 6% (w/v) trichloroacetic acid in a test-tube homogenizer. When metabolic experiments employed inulin, the slice was released to the fluid of the vessel in which it had been incubated, picked from this with a wire so that excess fluid was removed by touching the tissue on the side of the vessel, before placing in the tube. In the homogenizers, tissues were ground with a pestle, and after 15 min the homogenizer-tube was centrifuged. Portions of the supernatant were taken for determining Na and K with an EEL flame photometer.

For determination of lactic acid, 0·5- or 1-ml portions of incubation fluids were added to 4 ml $CuSO_4$ solution according to Barker and Summersen.[8] In manometric experiments, changes in oxygen pressure yielded μmoles O_2 absorbed per gram fresh weight of tissue, from which the respiratory rates quoted were obtained graphically.

Electrical stimulation. All tissues, whether receiving stimuli or not, were within electrodes. Manometric experiments employed the silver-grid electrodes of McIlwain

and Joanny[9] in vessels A, and other experiments, the quick-transfer electrodes.[6] Stimulating pulses were alternating in polarity and of exponential or rectangular time–voltage relationships, from apparatus of McIlwain and Rodnight[6] and McIlwain and Joanny,[9] respectively. In each case, electrical connections to the vessels to be stimulated were made momentarily after a few minutes' incubation, and the resistance at the vessel was checked by applying pulses below 0·5 V peak potential. After a chosen period (30–40 min) pulses of characteristics stated in the Results section were applied to the group of vessels to be stimulated, connected in parallel. After the chosen period of stimulation, the vessels were handled individually and at intervals of 30 or 60 sec, so that their tissues could be sampled either immediately after pulses stopped, or after chosen brief intervals, as described in the individual experiments.

RESULTS

Respiratory and glycolytic responses to stimulation

In concentrations between 10 and 500 μM the substances of Fig. 1 were without action on the respiration of cerebral cortical tissues in absence of stimulation (Table 1; Fig. 2). On electrical stimulation, however, the tissues became sensitive to 10 μM or

TABLE 1. RESPIRATION AND SODIUM OF CEREBRAL TISSUES TREATED WITH DRUGS AND STIMULATING AGENTS

Slices of guinea pig cerebral cortex were examined in glycylglycine-buffered media in manometric vessels. Pulses: of alternating polarity, rectangular time–voltage relationships, 7·5 V peak potential, 0·4 msec duration, and applied at 10/sec; KCl, to 50 mM. Each agent was applied after the tissue had incubated 40 min at 37 , and respiration was measured for the following 50 min, after which the tissues were transferred through sucrose and extracted for analysis for Na. Values are followed by S.D. and, in parentheses, number of values when data are available; other values are the mean of 2 or 3 determinations.

Added drug (μM)	Respiratory rate (μmoles O_2/g per hr) with applied agents			Sodium after KCl addition (μequiv./g)
	None	Rectangular pulses	KCl	
None	63 ± 3 (4)	105 ± 5 (4)	118 ± 6 (4)	131 ± 5 (4)
Haloperidol, 45	61 ± 3 (4)	60 ± 4 (4)	98 ± 7 (4)	148
Haloperidol, 500	62	56	68	162
Anisoperidone, 45	58	65	—	—
Anisoperidone, 500	63	65	—	—
R1636, 45	58	59	—	—
R1636, 500	61	61	—	—

less of each agent. Concentration-action relationships were examined most fully (Fig. 2) with pulses of exponential voltage–time relationships and of characteristics a little beyond the minima necessary for maximal response. These increased respiratory rates by 90% in the absence of added agents, and most of the substances examined were capable of eliminating this increase; haloperidol was not outstanding in this respect.

Tissue respiration increased by pulses of rectangular time–voltage relationship was also depressed by haloperidol and by the two analogues examined (Table 1). In a few experiments respiration was increased not by electrical stimulation but by making

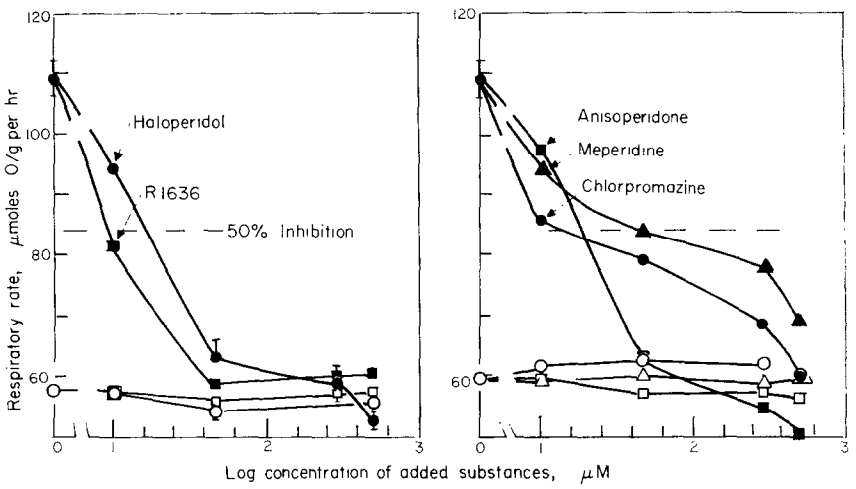

FIG. 2. Inhibition of respiratory response to stimulation, by haloperidol and cognate substances. The ordinates give respiratory rates of guinea pig cerebral cortex before (open points; added drugs named beside the correspondingly shaped points above) and during (filled points) the application of electrical pulses of exponential time–voltage relationships, peak potential 10 V, time-constant 0·4 msec, at 100/sec. Vertical lines extend from the points for distances corresponding to the S.D.; 4–6 observations.

media 50 mM in potassium salts. In absence of added agents this also increased respiration by 90%, and this increase was diminished by haloperidol. A markedly higher concentration of haloperidol was, however, required for a given degree of inhibition, in the KCl-treated tissues (Table 1).

The lactate accumulating on incubation in glucose-containing media was increased by electrical stimulation, and this increase was diminished by haloperidol (Table 2).

TABLE 2. HALOPERIDOL AND THE POTASSIUM, SODIUM, AND LACTATE FORMATION OF GUINEA PIG CEREBRAL CORTEX

Tissue slices were cut without contact with fluid, weighed, and placed in bicarbonate-glucose media at 37° containing 1% of inulin, mounted in quick-transfer electrodes and incubated in beakers containing 5 ml portions of the medium for 80 min. The vessels indicated contained 0·28 mM-haloperidol and all were equilibrated with 5% CO_2 in O_2. Electrical pulses of exponential time–voltage relationship, alternating, of peak potential 7·5 V and time-constant 0·4 msec, were applied to the vessels indicated during the last 50 min incubation, after which tissues were quickly released, briefly drained, and taken for analysis for Na, K, and inulin; media were sampled for determining lactic acid and inulin. The Table gives mean values followed by the S.D. when appropriate, and by the number of slices (in parentheses).

Substance	Unstimulated tissues		Stimulated tissues	
	No addition	Haloperidol	No addition	Haloperidol
Potassium content (μequiv./g tissue)	73 (3)	75 (3)	61 ± 3 (6)	70 ± 4 (6)
Sodium of non-inulin space (μequiv./g tissue)	38 (3)	40 (3)	44 ± 5 (6)	36 ± 4 (6)
Lactic acid accumulating in fluid (μmoles/g tissue)	82 (2)	82 (2)	176 (2)	101 (2)

Sodium and potassium content

The electrical stimulation which increased the respiration of normal cerebral tissue resulted also in loss of tissue potassium and gain of intracellular sodium[7] (measured as sodium which is not in the inulin space). A concentration of haloperidol which prevented the respiratory response to stimulation, also largely inhibited the ion changes (Table 2).

Three substances related in structure to haloperidol were compared with it and with chlorpromazine, under the conditions employed in Fig. 2. The tissues were sampled 5 min after stimulation ceased, and their total Na, K, and Cl content determined. During the 5 min, appreciable recovery of Na and K content occurred[7] and in most instances the ion content of tissues exposed to the added substances did not differ from that of tissues without addition, which had been similarly treated (Table 3; Fig. 3). Exceptions to this were tissues which had been exposed to electrical stimulation in the presence of the higher concentrations of haloperidol and chlorpromazine, between 45 and 500 μM, where the tissue sodium greatly increased (Fig. 3). This was

TABLE 3. SODIUM, POTASSIUM, AND CHLORIDE OF CEREBRAL TISSUES ELECTRICALLY STIMULATED IN THE PRESENCE OF CHLORPROMAZINE AND HALOPERIDOL

Experiments were carried out under the conditions of Fig. 2; all data refer to tissues which had received electrical stimulation for 40 min and were sampled 5 min after stimulation ceased.

Added agent (μM)	Tissue content (μequiv./g)		
	Potassium	Sodium	Chloride
None	40 ± 2 (10)	130 ± 4 (10)	114 ± 4·5 (10)
Chlorpromazine, 43	41 (2)	127 (2)	120 (2)
Chlorpromazine, 280	36 ± 3·5 (4)	158 ± 5 (4)	156 ± 5 (4)
Chlorpromazine, 500	23 (2)	201 (2)	189 (2)
Haloperidol, 280	40·5 (3)	159 (3)	143 (3)
Haloperidol, 500	24 ± 6 (4)	205 ± 13 (4)	172 ± 7 (4)

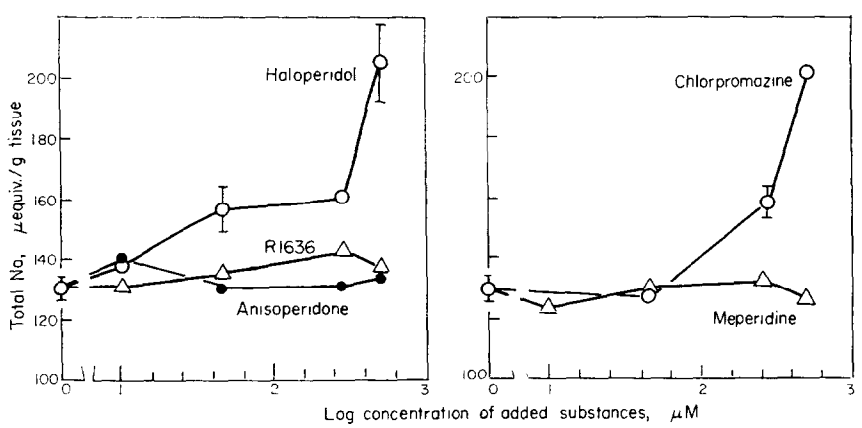

FIG. 3. The sodium content of guinea pig cerebral cortical tissues after incubation and electrical stimulation in the presence of added substances, examined and expressed as described in Fig. 2.

accompanied by an increase in chloride, while the potassium content was normal or diminished (Table 3).

DISCUSSION

Appreciable selectivity has been displayed in the action of the substances of Fig. 1 on isolated cerebral tissues. The substances were without effect in the concentrations examined (up to 500 μM) until the tissue was electrically stimulated, when it became sensitive to each of the compounds at 10 μM. Tissue respiration could be increased to a similar extent by addition of potassium salts, but was not then as sensitive to the added compounds as was that of electrically stimulated tissue.

The ion movements accelerated by electrical stimulation presumably prompt the additional respiration observed, and the potassium content of the stimulated tissue was also found to be sensitive to the added substances. A basis for their acting centrally can thus be presumed to be given by actions analogous to those seen in the isolated tissue. Basis for the particular type of behaviour which, *in vivo*, results from central action is understandably less likely to be exhibited by examining one category of isolated tissue, and it will be noted that in none of the properties observed at low concentrations of the drugs, was haloperidol very different from R1636 or anisoperidone. Collectively, however, these three were markedly more active than meperidine, as is indicated in Table 4, column (1). The tests of columns (3) to (6) of Table 4 show increasing selectivity of action in favour of haloperidol as more complex

TABLE 4. RELATIVE POTENCIES OF HALOPERIDOL AND RELATED SUBSTANCES

Data in columns (1) and (3) to (6) are reciprocals of ED_{50} values, expressed in terms of haloperidol = 1000. The ED_{50} values for columns (3) to (6) were from Janssen, van de Westeringh *et al.* (1959), Janssen and Neimegeers (1961), and P. A. J. Janssen, (personal communication).

Compound	(1) Response to stimulating tissue (Fig. 2)	(2) Accumulation of sodium in tissue (Fig. 3)	(3) Potentiation of phenobarbitone	(4) Righting reflex	(5) Rotating rod	(6) Avoidance-escape experiments
Meperidine	348	0	7	28	8	2
Anisoperidone	1000	0	118	63	89	4
R1636	1680	0	500	340	45	167
Haloperidol	1000		1000	1000	1000	1000
Chlorpromazine	1060	-	200	1630	174	67

central activities are examined. The only comparable specificity to haloperidol found in the present experiments is the increase in tissue sodium and chloride of Fig. 3 and Table 3. This, resulting from stimulating the tissue in the presence of the drug, differentiated haloperidol from anisoperidone, R1636, and meperidine. Moreover, it provided a biochemical point of resemblance to chlorpromazine, which haloperidol resembles in several of its central actions.[3, 10] Though relatively high concentrations of haloperidol and chlorpromazine were needed for action on the cerebral cortical

tissues examined, these concentrations acted only when the tissues were electrically stimulated, and then involved major changes in tissue electrolytes. Electrolyte distribution in intact rats is also affected by haloperidol (P. A. J. Janssen, personal communication). Such properties of the drugs thus merit further examination, for example in tissues from a number of parts of the brain.

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