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Comparison of Anticholinergic Potency to Psychotomimetic Action of Glycolate Esters. (32394)

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Ever since Ehrlich proposed the "key and analogy" to explain the action of many drugs, the concept of drug action has been viewed in a more systematic way. According to this theory, the biologic site for attachment of a drug consisted of a configuration analogous to the chemical structure of the drug. A substance such as acetylcholine was presumed to have a corresponding "receptor site" located at nerve endings and elsewhere in the nervous system. Atropine, on the other hand, exerted its effect by blocking the action of acetylcholine.

A molecule, such as atropine, can interact with a biological receptor site in various ways (1). The tertiary nitrogen, after protoration, can electrostatically interact with an anionic group on the receptor; while the carbonyl group can undergo hydrogen bonding with such functional groups as NH_2, OH, and SH. A van der Waal interaction can also occur between the aromatic group and lipophilic regions on the receptor site(2). The steric configuration of atropine and related substances must conform to certain chemical as well as spatial requirements for activity(3).

Such blocking agents have been extremely powerful tools in the elucidation of the mechanism and site of action of chemical transmitters. It is almost a tautology to assert that before a drug can produce an effect on a biological system it has to react, or interact, with a molecule of the system; consequently, there must be similarities in chemical constitution. There is a danger, however, in assuming that a drug like atropine can only act by blocking the action of acetylcholine, thereby relegating it to the role of a "physical obstruction".

The purpose of the present experiments was to present evidence that the action of many of the so-called anticholinergic agents upon the central nervous system is independent of acetylcholine. A group of glycolic esters of aliphatic and cyclic amines have been shown to possess hallucinogenic and anticholinergic properties(4,5). These studies have indicated 1) that anticholinergic potency did not always parallel psychotomimetic potency and 2) the administration of cholinergic agents, such as diisopropylfluorophosphate, did not antagonize the central actions of the glycolate esters. Besides contributing further evidence of this nature, the present study is concerned with extending the list and types of compounds in this series which possess psychotomimetic properties.

Methods. The compounds discussed were kindly provided by L. G. Abood. Anticholinergic action was measured by use of the superperfusion technique on the isolated smooth muscle preparation(6) and by measuring the degree of mydriasis in rats. The minimal concentration necessary to produce maximal mydriasis when a solution was applied directly into the rat's eye was used as the index for mydriatic effectiveness, while the ED_{50} (effective dose for 50% blockade of acetylcholine) was used to express anticholinergic activity. Hyperactivity was deter-

mined by means of a hyperactivity cage described elsewhere(7). Psychotomimetic effectiveness was evaluated on human subjects and the data were obtained from the article by Abood and Biel(2).

The swim maze, as developed by Kosman (8), was found to be a simple, effective way to evaluate the centrally mediated behavioral deficit produced by the glycolate esters. It consists of an I-shaped maze constructed of galvanized iron and has the overall dimensions of 100 × 75 × 20 cm (height). An adult albino mouse injected intraperitoneally with 1 mg/kg of the agent is placed in the water 5 minutes later, and both the time to swim the maze and the number of errors are recorded. Time scores are converted to the log X and the errors to $X\ 1/2 + (X + 1)^{1/2}$, only the error scores obtained in the fourth trial being used for evaluation. Since the swimming time was usually not affected by the drugs, their values were not recorded.

Results. Hyperactivity measurements in rats agree extremely well with psychotomimetic potency of the drugs in humans and disturbed swim maze performance in mice (Table I). Those agents failing to produce increased activity (greater than 100) are lacking in psychotomimetic action or effect on swim maze performance. The agreement between mydriatic potency and anticholinergic potency on the isolated ileum is fair, although there are notable exceptions such as compound XIII, atropine, and XVII. Anticholinergic potency, whether as determined on the ileum or pupil, does not correlate very well with either performance in the swim maze, hyperactivity, or psychotomimetic effectiveness. The most potent psychotomimetic agent of the series, I, is only moderately effective anticholinergically; while atropine and XVIII are extremely potent anticholinergically and weak psychotomimetically. One of the weakest anticholinergic agents, V, is among the more effective psychotomimetic agents, while one of the most effective anticholinergic drugs, XVII, is devoid of central action.

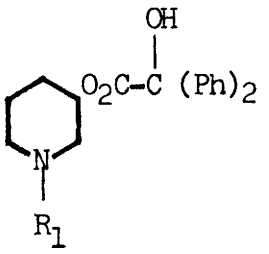
Discussion. These results indicate that there is not a strict correlation between anticholinergic and psychotomimetic potency in the present series of agents. Some of the weakest anticholinergic agents possessed significant central action while the converse was also true. There are two possible explanations for this disparity. Either the peripheral and central mechanisms of action of this series of agents are unrelated, or there must exist a

TABLE I. Comparison of Anticholinergic Potency with Central Action of Glycolic Acid Esters.

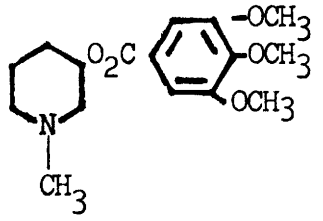
No.	R_1*	Anticholinergic: Ileum ED_{50}†	Anticholinergic: Mydriasis‡	Hyperactivity§	Swim maze‖	Phychotomimetic potency¶
I	$CH_2 \cdot Ph$	1.1×10^{-6}	1.5×10^{-4}	1018	4.0	5+
II	CH_3	2.2×10^{-7}	3.0×10^{-4}	1010	3.8	5+
III	$CH_2CH_2 \cdot Ph$	2.0×10^{-7}	1.5×10^{-2}	309	3.8	4+
IV	CH_2CH_2OH	9.0×10^{-8}	3.0×10^{-4}	300	3.6	3+
V	CH_2Ph	3.0×10^{-6}	2.5×10^{-3}	216	3.0	2+
VI	——	3.0×10^{-6}	3.0×10^{-2}	214	3.2	2+
VII	$C_2H_4NHN(CH_3)_2$	1.6×10^{-8}	1.5×10^{-2}	212	3.0	2+
VIII	$CH_2CH{=}CH \cdot Ph$	3.0×10^{-6}	3.0×10^{-2}	198	3.2	N.T.
IX	$C_2H_4OCH_2 \cdot Ph$	1.0×10^{-6}	3.0×10^{-2}	184	2.8	2+
X	——	6.0×10^{-6}	3.0×10^{-2}	164	3.0	N.T.
XI	——	3.2×10^{-6}	1.5×10^{-2}	164	2.7	2+
XII	$CH_2(CH_2)_2N(CH_3)_2$	3.0×10^{-6}	1.5×10^{-2}	153	2.7	2+
XIII	$CH_2CH_2N(CH_3)_2$	6.0×10^{-7}	1.5×10^{-2}	145	2.7	2+
Atropine	——	6.0×10^{-8}	3.0×10^{-4}	140	2.8	+
XIV	$CH_2 \cdot Ph \cdot CH_2Br$	2.0×10^{-6}	3.5×10^{-2}	139	2.6	N.T.
XV	$CH_2CH{=}CH_2$	2.0×10^{-6}	3.0×10^{-4}	75	2.8	+
XVI	$CH_2CH{=}CH \cdot Ph$	2.2×10^{-6}	1.0×10^{-2}	56	2.8	N.T.
XVII	$C_2H_4N(CH_3)_2$	8.6×10^{-8}	1.0×10^{-2}	55	2.6	0
XVIII	$CH_2CH_2NC_5H_{10}$	2.4×10^{-7}	1.0×10^{-2}	32	2.8	N.T.
XIX	$CH_2CH_2NC_4H_8O$	1.2×10^{-6}	1.0×10^{-2}	13	2.6	0
Saline	——	——	——	15	2.7	—

* All compounds for which R_1 is given have the "General Formula." Otherwise the chemical structure is as indicated. Ph = phenyl.

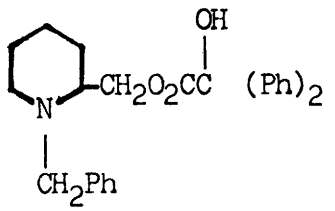
"General Formula"



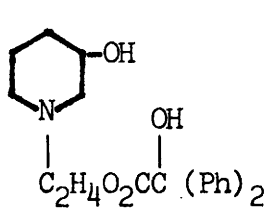
X



VI



XI



† ED_{50} is the molar concentration in 0.5 ml of Ringer's solution necessary to produce 50% inhibition of acetylcholine-induced contraction using the superperfusion technique.

‡ Mydriasis refers to minimal molar concentration necessary to produce maximal mydriasis.

§ Hyperactivity is expressed in terms of number of cage oscillations per minute.

‖ Swim maze values are expressed as errors on fourth trial (see text).

¶ Psychotomimetic data obtained from Abood and Biel(2); 5+ indicates maximal effect and 0 no effect; N.T. = not tested.

differential penetration of the agents into the brain and peripheral structures. There is little reason to believe that any permeability barrier to the agents exists within the ileum.

As has been demonstrated by Abood and co-workers(2,4), the central actions of the compounds were not significantly influenced by the administration of some cholinomimetic drugs particularly diisopropylfluorophosphate. Other cholinomimetic substances such as nicotine, arecoline, and carbamoyl choline were ineffective in counteracting or preventing the central actions of the piperidyl glycolates in rats, while at the same time reversing to a considerable extent the peripheral action of the agents. On the other hand, certain cholinesterase inhibitors such as tetrahydroaminoacridine and physostigmine will reverse the effect of the glycolate esters on performance in the activity cage and swim maze (9). It is not known to what extent this antagonistic action is due to cholinesterase inhibition but since many centrally active cholinomimetic drugs are ineffective as antagonists, it is difficult to relate the antagonistic action entirely to cholinergic mechanisms.

It is conceivable that the present group of compounds as well as anticholinergic agents in general may be acting directly upon receptor sites within the brain. The fact that the compounds are extremely potent anticholinergic agents as determined peripherally, would suggest that the receptor sites are cholinergic in nature. If one were to postulate an anticholinergic mechanism for the central action of the piperidyl glycolates, it would be necessary to assume the existence of an involved cholinergic mechanism for brain function, an assumption not without great difficulties(10). With regard to smooth muscle receptors there unquestionably exists a specificity for acetylcholine insofar as it induces muscle contracture. Conclusive evidence that acetylcholine has a physiological role in smooth musculature is, however, lacking.

It, therefore, appears likely that the piperidyl glycolates and related so-called "anticholinergic" agents are at least in part acting directly on the central nervous system. The so-called "cholinergic" receptor sites comprise a specific geometric pattern and all substances possessing the correct configuration will react

with this site. Similarly, another hallucinogenic substance, LSD, which is a serotonin antagonist at smooth muscle receptors, may also be acting directly on the central nervous system. The receptor sites have a configuration permitting interaction of substances resembling serotonin. Serotonin, like acetylcholine, serves only as a model for specific receptivity. Whether or not the biogenic amines play a role as chemical transmitters in some organisms is irrelevant to the present discussion. The receptor sites themselves, or "locks", are of primary importance; noradrenalin and histamine, as well, serve only as examples of "keys". The number of such sites within the nervous system exhibiting specificity may be very great as evidenced by the variety of pharmacological agents acting upon the nervous system. There may be innumerable endogenous agents, including polypeptides, which may be exerting an action at such sites.

Although the biogenic amines continue to be of immense importance to the chemist in designing new drugs, the biologists have used them, often indiscriminately, in the formulation of untenable theories of chemical transmission and drug action. Considerably more attention must be devoted to understanding the nature of the receptor site; and with the great advances being made in protein chemistry, this problem should be approached.

Summary. The anticholinergic action of a group of glycolic acid esters was compared with their psychotomimetic potency in humans and their efficacy in producing centrally mediated behavioral disturbances in rats and mice. Although all of the effective psychotomimetic agents were potent anticholinergic agents, the agreement between the two frequently did not hold. The findings are in support of the idea that the glycolate esters may, at least in part, be acting directly at synaptic receptor sites and not necessarily by cholinergic blockade.

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Some Chemical and Physical Characteristics of Purified Beta-Inhibitor From Bovine Serum. (32395)

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Following the initial work of Hirst(1) attempts to purify and characterize beta-inhibitor have been the goal of numerous studies (2-15). Although much progress has been achieved in these investigations, there are conflicting reports concerning the nature of beta-inhibitor. The present study was undertaken in the hope of delineating more precisely the physical and chemical properties of beta-inhibitor.

Materials and methods. Virus: The virus used throughout these studies was the Seerey strain of Type A Prime influenza virus as described by Briody *et al*(16).

Titrations: Titrations of hemagglutination (HA) and hemagglutination-inhibition (HI)