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III. Some New Approaches to Studying the Mode of Action of Central Nervous System Poisons*

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In attempting to explain the mechanism of action of any agents upon the central nervous system (CNS), a number of factors must be taken into consideration. If a particular agent is found to be an inhibitor (or activator) of some enzyme system, it is essential to explore the structural homologues and analogues for the same activity. Such structure–activity studies may not only aid in understanding the nature of the reactive chemical groups involved, but may also help to define the nature of the chemical receptors or reactive sites within the nervous system.

One of our major interests during the past few years has been the investigation of the properties of a group of piperidyl glycolates with psychotomimetic properties.[1–3] In the course of exploring some hundred structural analogues in this series, a number of interesting structure–activity relationships have emerged which are not only of practical significance in the development of new psychotropic agents, but which have pointed the way to an understanding of the mechanism of action of the agents upon the CNS.

Pharmacologically, the piperidyl glycolates are potent anticholinergic agents, and are thus related to acetylcholine. A three-dimensional model of acetylcholine can be compared with an agent such as *N*-methyl-3-piperidyl benzilate (I) (Fig. 1). The two reactive sites on the molecules are the cationic nitrogen of the amino alcohol and the carbonyl group of the acid which is capable of undergoing hydrogen bonding. The actual chemical difference between cholinergic and anticholinergic agents is relatively slight, although resulting in completely antagonistic pharmacological

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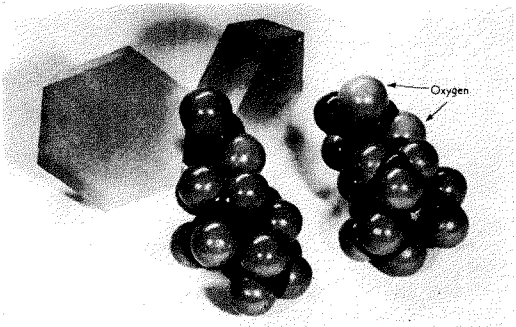


Fig. 1. Fisher–Taylor–Hirschfelder chemical models of *N*-methyl-3-piperidyl benzilate (I) (left) and acetylcholine (right). The two oxygen atoms in acetylcholine are so indicated. The plastic hexagons represent phenyl groups

properties. As the aliphatic chain of the amino alcohol residue increases, the cholinergic potency diminishes greatly (Table I).

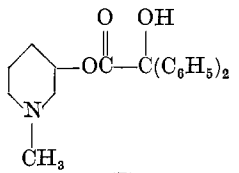
Table I. Properties of some esters of acids and amino alcohols[a]

Acid	Amino alcohol $R=N(CH_3)_3$	Cholinergic potency	Anticholinergic potency
Acetic	$-(CH_2)_2R$	100	
Acetic	$(CH_2)_2R$	0·1	
Acetic	$CH_2C(CH_3)_2R$	0·02	
Isovaleric	$CH_2C(CH_3)_2R$		1
Allylisopropionic	$CH_2C(CH_3)_2R$		10
Tropic	$CH_2C(CH_3)_2R$		200
Benzilic	$CH_2C(CH_3)_2R$		2000

[a] Cholinergic potency was evaluated on the basis of hypotension in the anaesthetized cat, anticholinergic potency on the isolated rat ileum. The relative potency is given.

If the amino alcohol remains unchanged, increasing the chain of an aliphatic acid will result in not only a diminution of cholinergic

activity, but also an increase in anticholinergic potency. As the acid strength increases, in other words, the ester is converted from a cholinergic to an anticholinergic agent. The presence of one aromatic (tropic ester) or two aromatic (benzilic ester) groups results in a 200- and 2000-fold increase, respectively, in anticholinergic activity in comparison with the isovalerate ester. The presence of aromatic residues on the acid not only increases acidity but makes the ester more lipophilic. With two aromatic groups apparently optimal anticholinergic activity is achieved in this series. *N*-Methyl-3-piperidyl benzilate (I) is not only one of the



(I)

most potent anticholinergic agents but is also among the most powerful psychotomimetic agents studied.[1]

At this point, it would be convenient to consider some of the chemical and physical factors of molecules involved in their interaction with biochemical systems. Some of the more important factors relating primarily to biogenic amines are given in Table II. With regard to *N*-methyl-3-piperidyl benzilate, the primary reactive site is the piperidine nitrogen. Modification of the charge on the nitrogen, e.g. by substituting alkyl groups on the

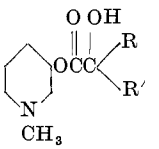
Table II. Factors influencing action of CNS drugs

Chemical	Physical
Cationic charge	Steric hindrance
Magnitude	Lipophilic–hydrophilic balance
Size	Planarity
Anionic influence	Secondary bonds
Reactivity	
Oxidation–reduction	van der Waals forces
SH	
Antimetabolite	
Chelation	

ring, results in a diminution of both anticholinergic and psychotomimetic potency. As indicated previously, anionic influences can greatly modify the action of aminoalkyl esters. The influence of many physical factors is also clearly evident in this series.

As long as at least one aromatic residue is present and the other aromatic group is replaced with cycloalkyl, the psychotomimetic potency is retained and even enhanced (see Table III). The

Table III. Effect of phenyl replacement on psychotomimetic and anticholinergic potency of piperidyl glycolates



	R	R′	Anti-cholinergic ED_{50}[a]	Psycho-tomimetic potency[b]	Duration, h
336	phenyl	phenyl	0·003	4	5
328	phenyl	cyclohexyl	0·01	4	12
8026	phenyl	cyclopentyl	0·003	5	18
344	phenyl	thienyl	0·001	3	1
PC-3	*o*-Cl-phenyl	*o*-Cl phenyl	0·5	1	1
BJ-1	*m*-Cl-phenyl	*m*-Cl phenyl	0·1	0	—
BS-1	*m*-CH_3-phenyl	*m*-CH_3 phenyl	0·5	0	—
330	phenyl	propyl	0·02	1	2
PC-1	fluorenyl		0·005	4	3

[a] ED_{50} refers to drug concentration necessary to block 50 per cent an acetylcholine-induced contraction of isolated rat ileum.

[b] Psychotomimetic potency was determined in human subjects on the basis of the following indices: hallucinations, delusions, confusion, degree of contact and communication, performance of tasks and psychological tests; maximal effect designated by '5', no effect by '0'.

phenylcyclopentyl glycolate derivative is even more potent than the benzilate, while at the same time it is more lipophilic. Lipoid affinity appears to be a primary requisite for entry of a compound into the central nervous system. As alkyl and halide substituents are incorporated into the phenyl rings, anticholinergic activity diminishes greatly while psychotomimetic potency vanishes. In addition to other influences the presence of such groups contributes

to steric hindrance in the aromatic structures, which in turn prevents coplanarity. Fixation of the two phenyl rings in a coplanar configuration, as in the fluorenyl derivative (PC-1), restores both psychotomimetic and anticholinergic activity. In referring to Fig. 1, it can readily be imagined how a coplanar configuration would facilitate attachment and adherence to a receptor site.

Possible Mode of Action of Piperidyl Glycolates

The next point to consider is the mode of action of this particular group of compounds. In the course of investigating the distribution of tritium-labelled *N*-ethyl-3-piperidyl benzilate within the central nervous system,[4] it was found that the drug was localized predominantly in a cytoplasmic granular fraction consisting largely of mitochondria. Whittaker[5] is of the opinion that this particular fraction—obtained by the differential centrifugation of brain homogenates prepared with isotonic sucrose—contains synaptic vesicles to which are bound not only acetylcholine but also serotonin and norepinephrine as well. Not only have attempts on our part to demonstrate the presence of synaptic vesicles in our 'mitochondrial fractions' been unsuccessful, but, in employing the techniques of Whittaker to separate the synaptic vesicles from the mitochondria, it was found that over 75 per cent of the mitochondrial respiratory metabolism was destroyed. It would appear, therefore, that the piperidyl glycolates are actually bound to mitochondria.

As a result of these studies concerning the binding of the piperidyl glycolates to mitochondria, it was desirable to determine in what respects the drugs were affecting mitochondrial function and metabolism. Through the use of adult neurons grown in tissue culture, Geiger[7] has been able to demonstrate that these compounds produce a considerable increase in the movement of cytoplasmic granules, particularly in the vicinity of the Nissl substance and in the dendritic and axonal endings. By measuring optical changes of mitochondrial suspensions, it can be shown that piperidyl glycolates bring about alterations in the structure of the mitochondria. Using orthophosphate P^{32} as a tracer, we found an appreciable activation of phospholipid turnover of rat brain

and liver mitochondria with these agents. The turnover is particularly marked in the diphosphoinositide and the lecithin fraction. This group of agents was without effect on a wide variety of enzymes, including components of the electron-transport scheme, esterases, phosphatases, and oxidases. The binding of the piperidyl glycolates to mitochondria can be interfered with by many neuropharmacological agents, such as chlorpromazine, reserpine, acetylsalicylate, meprobamate, and 1,2,3,4-tetrahydro-9-aminoacridine. This latter compound (THA) has been found to be a specific antagonist to the psychotomimetic action of the piperidyl glycolates in humans.[6] These findings with mitochondria, along with the observation that mitochondria contain the cholinergic receptor sites, strongly indicate that the action of the piperidyl glycolates in biological systems is occurring at this level of the cell. Indeed, studies with tissue culture neurons indicate that a wide variety of neuropharmacological agents, including convulsants, sedatives, and narcotics, are acting on such subcellular granules.

Action of Drugs at the Mitochondrial Enzyme Level

In view of previous studies showing the effect of aromatic structures on the inhibition of oxidative phosphorylation of brain mitochondrial systems, it was desirable to determine whether the piperidyl benzilates, which possess two aromatic rings, would also be inhibitory. As indicated previously, however, it was found that these compounds fail to act not only on this but also on many other enzyme systems tested. The reason for this difference was worth investigating from the point of view of chemical constitution. Various compounds in which two aryls converge on a dimethylaminoethyl group were compared for their effect on respiratory metabolism (Table IV). In those antihistaminics possessing benzhydryl and diphenylamine structures, there was considerable inhibition of oxidative phosphorylation of brain mitochondria at concentrations of 10^{-5}M. However, when an ester-type linkage occurred between the alkylamine and the diphenyl-substituted acid, there was no longer any effect on oxidative phosphorylation. At the same time, these esters

Table IV. Inhibition of oxidative phosphorylation by diphenyl derivatives

Structure $R=(CH_3)_2NCH_2CH_2$—	Example	Property	P/O inhibition[a]
H $RC(Aryl)_2$	Chlorprophenpyridamine Analgesics	antihistaminic	4+
$RN(Aryl)_2$	Tripellenamine Diethazine	antihistaminic	3+
H $ROC(Aryl)_2$	Diphenhydramine	antihistaminic	3+
OH $ROOCC(Aryl)_2$	Benactyzine	anticholinergic	0
H $ROOC(Aryl)_2$	Adiphenine	anticholinergic	0
$ROOCN(Aryl)_2$	–	anticholinergic	0

[a] Oxidative inhibition was determined on rat brain mitochondria according to methods described elsewhere.[6]

became relatively weak antihistaminics and potent anticholinergic or ganglioplegic agents.

The effect of a second phenyl group in influencing metabolic inhibition is apparent in examining the effect of the compounds in Table V. Compounds such as phenethyldimethylamine have but

Table V. SA studies with diphenyl derivatives

$R=CH_2CH_2N(CH_3)_2$	P/O	Hx[a]	Ax[b]	CN[c]
PhR	1	0	0	2
PhOR	0	0	0	2
PhNHR	2	1	0	5
$(Ph)_2CHOR$	10	10	5	10
$(Ph)_3NR$	8	5	1	10
$(Ph)_2COOR$	0	1	10	0

[a] Antihistaminic action where the maximal effect is arbitrarily 10.
[b] Anticholinergic potency with the maximal effect as 10.
[c] CNS depression.

a slight effect on oxidative phosphorylation, as do those having an oxy and imino linkage between the aromatic and alkylamino substituents. These compounds are rather weak antihistaminics

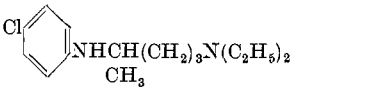
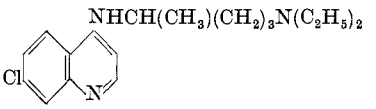
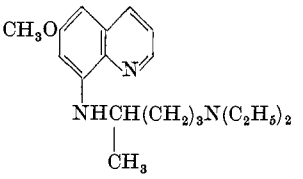
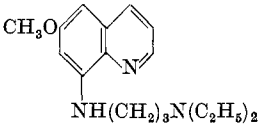
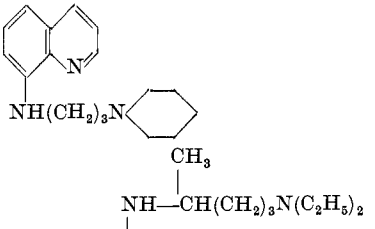
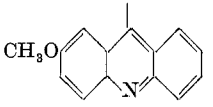
and possess no anticholinergic activity. They do, however, produce a certain degree of central nervous system depression. In the case of diphenyl congeners of the monophenyl derivatives, the situation is quite different. They are not only very potent inhibitors of oxidative phosphorylation, but are also potent antihistaminics and central nervous system depressants.

On the basis of these studies, it would appear that some correlation exists between the CNS depression produced by these agents and the metabolic inhibition. The anticholinergics, on the other hand, are devoid of any inhibition of respiratory metabolism and instead of being CNS depressants are potent stimulants. To what extent the so-called antihistaminic properties of substances such as diphenhydramine and tripellenamine are attributable to their inhibitory effect on neural metabolism remains to be seen. It is noteworthy that chlorpromazine, which is a potent antihistaminic as well as an inhibitor of oxidative phosphorylation, is an effective tranquillizing agent.

As indicated in a previous study, a wide variety of so-called diphenyl derivatives were effective inhibitors of respiratory metabolism, particularly oxidative phosphorylation.[8] In a group of antimalarials, where the active moiety is a dialkylaminoalkylimino substituent, it was also possible to demonstrate a relationship between the inhibition of oxidative phosphorylation and CNS depression (Table VI). The chlorphenyl derivative (VI-1) produced little CNS depression and effect on phosphorylation. Plasmocid (VI-4) (8-diethylaminopropylamino-6-methoxyquinoline) was an extremely potent inhibitor of oxidative phosphorylation and produced marked CNS depression. This compound, along with 8-(3-piperidinopropyl)aminoquinoline (VI-5) is of little value as an antimalarial because of the marked CNS effect. Plasmocid is known to produce a wide variety of CNS disturbances, including muscular rigidity, nystagmus, and lesions throughout the extrapyramidal and vestibular-cerebellar structure.[9] Chloroquin (VI-2) which is devoid of CNS effects, and is a weak inhibitor of oxidative phosphorylation, is among the more effective antimalarials.

On the basis of these findings, one might infer that in a given group of compounds possessing CNS action, relationships may exist between inhibition of respiratory metabolism and CNS

Table VI. Comparison of metabolic inhibition and CNS depression in a group of 8-aminoquinolines and related substances

		Name	P/O,[a] % inhib.	CNS depression[b]
VI-1		—	25	Mild
VI-2		Chloroquin	20	None
VI-3		Pamaquin	30	Mild
VI-4		Plasmocid	65	Marked
VI-5		—	70	Marked
VI-6		—	35	Mild

[a] Oxidative phosphorylation, the percentage inhibition indicated referring to a drug concentration of 5×10^{-5}M.

[b] CNS depression was measured by the techniques described in the text.

depression. This hypothesis is applicable certainly to the phenothiazine group of tranquillizers, as well as to a wide variety of antihistaminics. By no means does this imply that inhibition of oxidation or phosphorylation is a concomittant of CNS depression.

There is, however, reason to believe that a wide variety of anaesthetics and hypnotics, including the barbiturates and the volatile anaesthetics, affect respiratory metabolism in relatively small concentrations and could conceivably act in part by virtue of such inhibition. The mechanism by which the diphenyl-type compounds produce their inhibition of phosphorylation and oxidative metabolism is discussed in detail elsewhere.[8]

The scheme in Fig. 2 describes the various metabolic pathways involved in energy production and utilization, along with known

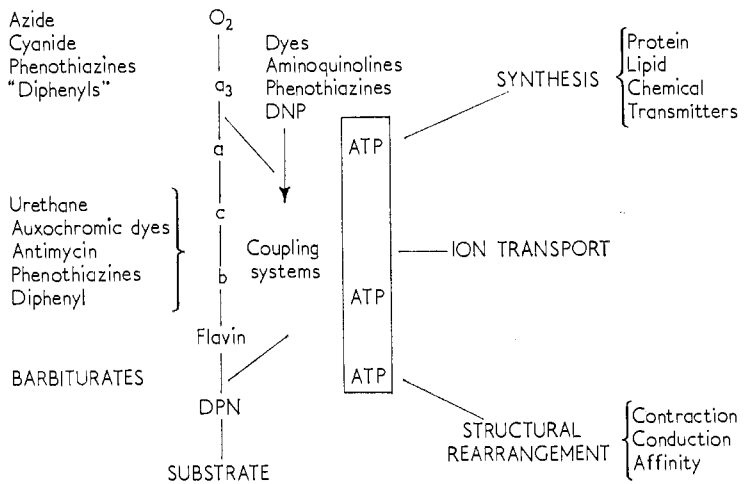


Fig. 2. Pathways of cellular energy production, coupling, and utilization and sites of drug interference

sites of drug action. The phenothiazines and the antihistaminics appear to act at two points in the electron-transport scheme, namely, at the level of cytochrome oxidase and between cytochrome b and cytochrome c. Substances such as the barbiturates are presumed to act between reduced DPN and the flavin enzyme. Metabolic inhibitors which interfere with the production of ATP can directly affect function by cutting off the main fuel supply for the cell. There are, however, other ways in which drugs can act, and this is in the link between ATP and function. As mentioned previously, with regard to the possible mode of action of the piperidyl glycolates, drugs can interfere with cellular structure and per-

meability or they can influence the synthesis of structural and functional elements such as the proteins, lipids, and chemical transmitters. There is still a third possibility, and that is that the drugs could conceivably inactivate or block certain receptor sites normally acted upon by endogenous chemical transmitters. The effects of the curare-like agents and the anticholinergics on the peripheral nervous system are examples of this latter mechanism.

Conclusions

The objective of this discussion was primarily directed towards elucidating the mechanism of action of a particular chemical group possessing CNS properties. It is all too apparent, however, that we are far from any precise explanation of how agents such as the piperidyl glycolates are able to produce hallucinations, confusion, and other psychotomimetic effects. Even if it were possible to postulate an anticholinergic mechanism to explain the CNS action of this class of compounds—and such evidence is largely lacking—the fundamental problem linking the biomolecular interactions to the behavioural responses remains unsolved.

In view of the functional complexity of the CNS and its multifarious responsiveness to drugs, it has been necessary to postulate the existence of 'receptor' or 'acceptor' sites within the nervous system, each with its peculiar chemical configuration and behavioural correlation. The localization of various neurohumoral amines in certain structures within the CNS is strong evidence in support of this concept. Such agents as 5-hydroxytryptamine, norepinephrine, dopamine, and histamine appear to be concentrated in the caudate nucleus, hypothalamus, and other structures of the limbic system. Insofar as this portion of the brain is concerned with emotion and effect, as well as visceral regulation, and in view of the fact that many of the psychotropic agents either mimic, or interfere with, the action of the neurohumoral amines on the autonomic nervous system, there has been a tendency to explain their central action by the same mechanism. Consequently, an agent such as LSD is presumed to act centrally by blocking the action of 5-hydroxytryptamine just as it blocks the contractile response of smooth muscle to this same neurohumoral agent. Similarly, an agent such as *N*-methyl-3-piperidyl benzilate,

which is an extremely potent antagonist to the acetylcholine-induced contraction of smooth muscle, might be presumed to exert its central action by cholinergic blockade.

The objections to this approach to explaining the CNS action of the psychotropic agents have been discussed in detail elsewhere.[3] Even though applicable in rare instances, this postulated mechanism is based on the untenable assumption that the so-called neurohumoral amines have a definite action in the CNS. The possibility must be considered that these amines merely define the chemical configuration of the receptor site, and that agents such as LSD and the anticholinergic psychotomimetic agents exert a direct action at such sites.

Although it is conceivable—and, indeed, highly probable—that there are intrinsic chemicals normally active at such sites, they need not be the same as the chemical transmitters active in the peripheral nervous system. A large number of additional amines, polypeptides, nucleotides, and lipids present in the nervous system have been shown to act on neural and muscular tissues. The recent work of Honegger and Honegger[10] showing the presence of various volatile amines in the central nervous system of mammals is particularly noteworthy in this respect. In addition to certain aliphatic amines, they reported the presence of pyrrolidine and piperidine to the extent of 30 μg/kg and dimethylamine to the extent of 550 μg/kg. Recently, we have shown that piperidine itself is an extremely interesting new type of psychotropic agent effective in the treatment of paranoid schizophrenics.[11] The interesting possibility presents itself that piperidine or some piperidine-like substances may be intrinsically active in the central nervous system as a psychotropic agent. Slight alterations in the chemical structure of piperidine cause considerable diminution of the psychotherapeutic action of the compound, while at the same time increasing the curare-like action and the toxicity (Table VII). These findings emphasize the need for further search for endogenous neuropharmacological agents, as well as for the development of effective methods for their screening. The effort to elucidate the nature and action of such agents must continue to proceed at an ever increasing pace.

Nonetheless, the more neglected hypothesis that psychotropic agents may exert a direct action at the receptor sites deserves

Table VII. Properties of *N*-substituted piperidines

Substituent	Central depression	Curarization	Toxicity
Piperidine	↑ increase	↓ increase	↓ increase
2-CH_3			
2,6-$(CH_3)_2$			
2,4,6-$(CH_3)_3$			
2,6-$(CH_3)_2$; 4-C_2H_5			
2,6-$(CH_3)_2$; 4-C_3H_7			

greater attention. The present discussion has endeavoured to describe certain approaches to this problem which have yielded new interesting and useful psychotropic agents.

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