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Qualitative Identity of Cerebral Neuronal Membrane Actions of 5HT LSD, and CPZ

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Extra- and intracellular recording of the cerebral cortical actions of close-arterially injected serotonin (5HT) and LSD in the cat shows them to be powerful synaptic inhibitors. They are specifically and differentially blocked by chlorpromazine (CPZ). The membrane parameters including spike generation, polarization, transmembrane conductance, and IPSPs show that all three produce qualitatively *identical changes, which must, therefore, be presumed to act on the same receptors with block by CPZ taking place because of competitive inhibition. The relation of these neuronal membrane findings to the characteristic actions of LSD and CPZ in mental disturbance is considered in relation to a general concept of cerebral synaptic dysfunction.*

INTRODUCTION

A full understanding of integrative actions requires detailed knowledge of the relevant changes in the constituents units. Ultimately the focus must be on the single cell and here the initial, crucial changes must take place at the interphase with its environment (Marrazzi, 1975). In this case, this means the cerebral neuronal cell membrane.

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Since drugs can most readily act on the nervous system by influencing or modifying the neurohumoral chemical environment of the neuronal cell, it becomes instructive to examine their effects on the cell membrane. We have already established on the basis of field potential changes (Marrazzi, 1961; 1962) that chlorpromazine (CPZ) can protect against the cerebral synaptic inhibitory actions of serotonin (5HT) and lysergic acid diethylamide (LSD) (Marrazzi, 1966; Halasz *et al.*, 1969) and, furthermore, that larger than protective doses of CPZ can themselves inhibit. This, along with the parallelism of the dose-response curves we have obtained for 5HT and CPZ individually and in combination, strongly suggests that CPZ blocks 5HT, as well as adrenergic amines (Ghouri *et al.*, 1973) and LSD, by preempting the receptors substituting a weak for a strong action, i.e., by competitive inhibition.

The adrenergic amines we have studied (Marrazzi, 1975; cf. Table VI-4) include adrenochrome, dopamine, norepinephrine, metanephrine, and DMPEA as well as the amine accumulators, amphetamine, ephedrine, and MAOI, all of which we found produced synaptic inhibition that was blocked by CPZ. Our data obtained chiefly from the cerebral cortex (also thalamus and brain stem) have been extended to the cerebellar cortex by Faingold and Marrazzi (1972; 1973) and more recently by Freedman (1977), who obtained block of norepinephrine-induced inhibition of spontaneous firing by another phenothiazine (fluphenazine). Likewise, dopamine striatal inhibition is blocked by CPZ in the cat, monkey, and rat (Siggins, 1978). Thus the *qualitative* similarity displayed in the pharmacological characterization of representative synapses in various areas of the brain leads to the search for similarity in the underlying membrane processes. The testing of the competitive inhibition hypothesis is an ultimate step in formalizing a significant explanatory generalization making membranes, i.e., those of their receptors, susceptible to whole families of substances, as with the adrenergic and indole amines, *ipso facto* vulnerable to competitive inhibition and so operating, in part.

METHODS AND RESULTS

We have studied antagonism at the unitary level, and, as shown in cortical extracellular unit firing in the cat in Fig. 1, we obtain protection against both 5HT and LSD by previous administration of CPZ, in doses small enough not to affect firing when given alone. All injections were close-arterial (intracarotid) in uniform (0.1 cc) volumes delivered in 1 sec. Antagonism was studied by noting the effects of the same test dose of agonist alone and when preceded (2 min) by CPZ. The records are representative of six similar experiments.

Male cats not over 2 kg were used. The initial preparation involving immobilization in a stereotaxic head holder and exposure of the cortex was carried out under sodium pentothal. The cat was then flaxedilized with supplements as required, and artificially respired through a tracheal cannula. Anesthesia

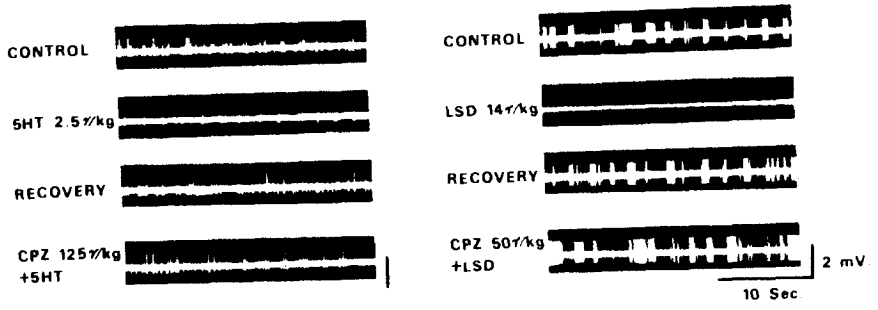

Fig. 1. Chlorpromazine block of serotonin and LSD. Extracellular unit discharge in pericruciate area of flaxedilized cat. Injection: ipsilateral common carotid artd artery.

was supplemented by lidocaine carefully and repeatedly applied to the cut edges of the scalp and neck, exposed muscles, and to the corneal surface. Agar protected the exposed pericruciate cortex. Bilateral pneumothorax and a pressure foot restraining the cortex were used to minimize respiratory and vascular pulsation. The microelectrode was inserted by a microdrive through a hole in the specially constructed metal pressure foot, which could be used to simultaneously record surface cortical potentials. Glass microelectrodes with a 1-μ tip size, filled with 3 M KCl were used for extra- and intracellular recording after high-gain differential amplification with a neutralized input in the case of the intracellular. The potentials were displayed on a dual-beam oscilloscope and also fed into a loudspeaker. The upper beam was used for ac amplification of the firing and the lower beam, connected in parallel, for dc amplification of the membrane potentials and the dc pulses that are proportional to the transmembrance resistance. IPSPs were most successfully evoked by stimulation of the ventrolateral thalamus.

The specificity of the CPZ action in differentially blocking 5HT and LSD but not other CNS synaptic inhibitors, such as γ-amino butyric acid (GABA) and glycine (Ghouri *et al.* 1973; Ghouri and Marrazzi, 1971, 1972) which are differentially blocked by bicuculline has led us to look for properties common to both the agonists and antagonist -- properties that might, indeed, define functional parameters of a common receptor moiety. Accordingly, we measured the cortical cell membrane parameters by an intracellular electrode placed in one of the sufficiently large cells that are readily found in the pericruciate area of the flaxedilized cat. These are presumably Betz cells, and, indeed, in initial explorations of this region we identified such cells by their response to antidromic stimulation of the pyramidal tract. Since this identification of cells large enough to accommodate a 1-μ microelectrode is not vital to the argument, it was not made a part of the subsequent routine procedure. We monitored the course of changes during the rise, plateauing, and fall of effective dosage, following the sharp gradient produced across the blood-brain barrier by an ipsilateral close-arterial (intracarotid) injection of the substances to be tested.

Fig. 2. 5HT, LSD, and CPZ inhibition of spontaneous unit discharge in pericruciate area – continuous monitoring. Hyperpolarization and transmembrane resistance of flaxedilized cat. Injection: ipsilateral common carotid artery.

As shown in the intracellular recording Fig. 2 (representative of 15 units), in which the spontaneous spike discharges are followed in the upper (ac) record simultaneously with the membrane potential in the lower (dc) record, 1 μg of 5HT or 5 μg of LSD per kg have *qualitatively* identical kinds of action. The reduction in spike discharge parallels the hyperpolarization and the transmembrane conductance measured by the height of the dc potential change induced on the injection of a brief nonstimulating pulse of 10^{-9} amp. As we have previously reported (Huang and Marrazzi, 1972a; 1972b) like Siggins *et al.*'s (1971) cerebellar results with norepinephrine, which we also confirm for the cerebral cortex, transmembrane resistance increases or the conductance decreases unlike the opposite change that occurs during the otherwise similar inhibition by GABA or glycine (Huang and Marrazzi, 1973). The 5HT and LSD doses chosen were small enough so that recovery could be demonstrated in the same cell (i.e., during the successful maintenance of an intracellular recording, as long as 3 min). The relatively short duration of the inhibition so obtained, slightly complicated in the case of LSD by the concentration in this region of the pulses used to measure transmembrane conductance, is followed by an increase or postinhibitory rebound, i.e., by a release phenomenon or disinhibition such as we have previously described (Marrazzi, 1975), before firing returns to control level in recovery.

A further striking finding is illustrated in the lower part of Fig. 2, which shows that CPZ produces a *qualitatively* similar set of phenomena. Protective doses of CPZ, as in the case of extracellular unit recording, prevented or reduced all the changes in membrane parameters that we demonstrated in the same cell for 5HT before administration of and after recovery from CPZ. However, to illustrate the similarity of the antagonist membrane actions to the more powerful ones of the agonists, we are using in Fig. 2 a much larger dose of CPZ – large enough to produce overt inhibition. The inhibition (15 sec) is followed, as with the agonists, by a further (40 sec) postinhibitory rebound, and then return to control level. The last line of Fig. 2 demonstrates that a local anesthetic effect of CPZ is not involved, since a ten-times larger dose of a local anesthetic, xylocaine, adequate to reduce the spike discharge, produces none of the other membrane changes.

DISCUSSION

Figure 3 summarizes the identity of the kind of membrane changes for both agonists and antagonist, including changes in IPSPs elicited by ventrolateral thalamic stimulation. The control, negative findings with a local anesthetic are given in the bottom line. The data (representative of similar results in 15 units in which the sequence was completed) clearly indicate that agonists and antagonist, this is, 5HT and LSD on the one hand and CPZ on the other, all three act on the same ionic mechanisms: channels or iontophores. Furthermore, since the

MEMBRANE CHANGES IN POSTSYNAPTIC INHIBITION BY DRUGS AND ITS BLOCKER

Drugs		↓ Spike #s	↑ Polariz.	↑ Resist.	↑ IPSPs
Agonists	5HT	+	+	+	+
	LSD	+	+	+	+
Antagonist	CPZ	+	+	+	+
Anaesth.	XYL	+	-	-	-

Fig. 3. Pericruciate pyramidal cell – intracellular recording, flaxedilized cat.

antagonist CPZ is clearly, itself, a weak inhibitor, as shown by the reduction in spike discharge and induction or enhancement of IPSPs by doses larger than simply protective ones, the ionic mechanisms measured becomes highly relevant. The competitive inhibition, shown by the present data, as well as by parallelism of the dose-response curves for the agonists and antagonist singly and in blocking combinations we have previously reported (Ghouri *et al.*, 1973), indicates strongly that both the agonists and the antagonist may be acting on the same trigger mechanism or receptor moiety.

The remarkable fact established, both in populations of neurons (extracellular recording) and in the individual constituent neurons (intracellular recording), is that the actions of the most potent mammalian cerebral neurohumoral inhibitor, serotonin, of the powerful hallucinogen, LSD, and the classical tranquilizer CPZ, are *qualitatively* identical, but with quantitative differences in that serotonin is extremely potent (1 μg), LSD highly potent (5 μg), and chlorpromazine has a weak (50 μg) serotonin- or LSD-like action. The membrane dynamics are thus supplying direct evidence of involvment of the same receptors and the identity of kind of actions elicited, as required by the mechanism of competitive inhibition. Accordingly, the preempting of a receptor by an antagonist, thereby substituting a much weaker for a strong action, results in block.

Thus the LSD which is a weaker inhibitor than 5HT can competitively inhibit it, while CPZ can competitively inhibit both 5HT and LSD. The same kind of actions on the same receptors also accounts, as we have previously pointed out (Huang and Marrazzi, 1972c), for the additive effects among the three. This is, for example, evident in the exacerbation of agonist action when the antagonist is exhibited in a dose that is larger than protective (Halasz *et al.*, 1969).

CONCLUSION

The relevance, clinical and experimental, of LSD and CPZ effects to mental disturbance, regardless of the extensive controversy as to whether there are, indeed, model psychoses – no less drug-induced ones – is inescapable. Without, then engaging in any such debate, it becomes rewarding to explore further the influence and mechanism of the action of these substances in modifying integrative function. This prime attribute of the nervous system is indeed compromised in mental disturbance and is manifest as deviant behavior. Thus, these experiments indicate that LSD and CPZ may act at the same receptor and that such receptors indeed are implicated as a site of psychopathology in schizophrenia. However, in view of the generality of these cerebral synaptic actions (Marrazzi, 1964; Marrazzi and Urquiaga, 1968), the process is far from being specific; and, in that sense, there is no "schizophrenic process" distinguishable from a synaptic dysfunction that presents itself as schizophrenia in some cases.

Pertinent to this is the synapatic neurohumoral imbalance we (Marrazzi, 1966; 1975) have considered to be the pathophysiology and again invoked by Berger *et al.* (1978), who refer to "altered balance between two or more neuroregulators" underlying schizophrenia, while Lasagna (1978) speaks of treatment of the "total disorder" rather than symptomatic therapy, and Bunney and Aghajanian (1978) offer, as an alternative to the currently popular dopamine hypothesis, that antipsychotics through dopamine neurons could correct "an *imbalance* caused by malfunction of *one or more non-dopamine* systems." [Italics are ours.]

The generality of the synaptic inhibitory actions described as well as the similar but not identical actions and therefore different group of receptors for GABA and glycine predicates that the representative kinds of cells we have sampled in cortex, thalamus, brain stem, and cerebellum are all possessed of the same qualitative complement of varied inhibitory as well as excitatory receptors (Marrazzi, 1973).

The data provide a neuronal membrane basis for a specific, competitive, and differential inhibition, and supply some of the membrane dynamics of a functionally characterized specific receptor.

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