Some Indications of Cerebral Humoral Mechanisms

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WHEN OTTO LOEWI was appointed Research Professor of Pharmacology at the New York University College of Medicine in 1940, the writer, then a member of the Department of Pharmacology, along with his fellow faculty members looked forward to Professor Loewi's coming with eager anticipation and considerable uncertainty as to the place of a European Nobel laureate in their midst. It is a glowing recollection and an invaluable educational experience, how quickly and surely the great man, who turned out, despite his recent reverses, to be so jovial, so keen, so distinctly democratic, and so humble in his desire to learn, became very much one of us and of American science.

In honor of Otto Loewi's eightieth birthday, which is being celebrated this year, the writer is happy to express his fond respects and to acknowledge his debt by tracing the relation of the work of his group to Loewi's bold and inspiring contributions and thinking.

With a masterful preparation (*1*), Loewi at once offered the first direct indication of humoral transmission at neuroeffector junctions and strongly suggested the cholinergic and adrenergic natures of the cardiac transmission phenomena. These bore a reciprocal relation to each other, paralleling the influence on the heart of its two controlling innervations (*2*).

Our success in identifying the similar situation at the neuroneuronal junctions or synapses of autonomic ganglia (*3–5*) has led us to ask whether analogous mechanisms can be detected at cerebral synapses. This is a brief review of the work along these lines going on in our laboratory.

We have found it particularly advantageous to study synaptic transmission *in situ* by delivering to the synapses selected for study impulses of known intensity, initiated at will by electrical stimuli to the presynaptic nerve, and recording the output from the synapses in the form of postsynaptic action potentials (*6, 7*). With the circulation preserved, this allows immediate and certain localization of the action of bloodborne substances to a particular group of synapses in a manner that can be readily quantitated. In this way we have been able to demonstrate in synapses of autonomic ganglia, both sympathetic (*6–12*) and parasympathetic (*13–15*), a cholinergic excitatory mechanism reciprocating with an adrenergic inhibitory one.

Since there seems every reason to regard the monosynaptic pathways in autonomic ganglia as representative of the simplest unit in the central nervous system, it was decided to devise an experimental situation in the brain that was as closely similar as possible. We have utilized three such preparations of varying complexity. The simplest and most closely similar to the ganglionic preparation is the two-neuron, monosynaptic, transcallosal pathway connecting one cortex to symmetrical points in the contralateral optic cortex of the cat (*5, 16–20*) (Fig. 1). By

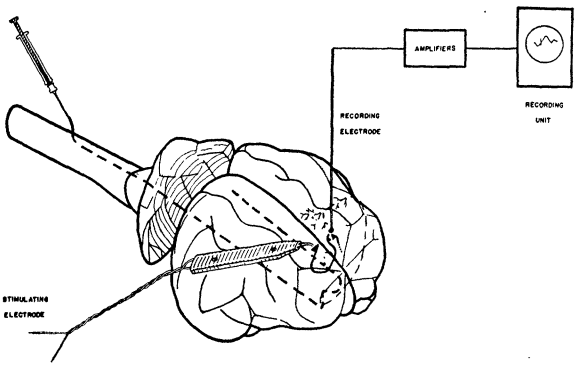

FIG. 1. Transparent model of the brain of the cat, showing intercortical pathway and cerebral blood supply.

stimulating one optic cortex and recording from the other in the cat, lightly anesthetized with sodium pentobarbital, it is possible to follow synaptic action. Owing to the manner in which currents flow in a large mass such as the brain (volume conductor properties), the input to the synapses in the pathway reflects itself as a surface positive wave in the potentials recorded from the cortical electrode overlying the postsynaptic cells. The latter become negative as soon as transmission has been effected and they are activated, thereby producing a surface negative wave at the recording electrode. Thus, a simultaneous electrical record of input and corresponding output from the synapse is available, so that a differential action, i.e., change in output without change in input, indicates directly, per se, that the observed change is synaptic in origin.

By inserting the stimulating electrode into the optic nerve or tract (*3–5*) and recording from the same cortical point, an additional synapse is traversed, the impulses now passing through the lateral geniculate body and by way of the optic radiations to the optic cortex. It has thus been possible to compare effects on polysynaptic and monosynaptic pathways, terminating at the same cortex. The effects on both preparations are identical, except for the lower threshold of the poly-

synaptic one, suggesting that in such chains the synaptic effects can cumulate.

To insure, on the one hand, adequate local concentrations of substances that, like acetylcholine, are rapidly destroyed and, on the other, to elicit actions confined initially to the ipsilateral[1] cortex, injections were often made into the common carotid artery on the side from which potentials were recorded, thereby approaching the advantages of a close arterial injection (Fig. 1). In this way acetylcholine (*5, 14*), in as little as 1 gamma per kilogram, can be shown to produce a clear-cut enhancement of the response or synaptic output elicited by a test input of submaximal intensity (Fig. 2). Doubling the amount produces a depression

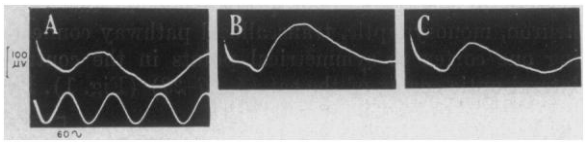

FIG. 2. Cerebral action of acetylcholine. Potentials evoked in optic cortex by stimulation of symmetrical point in opposite cortex. In this and subsequent figures negativity is upwards. Acetylcholine 1 γ/kg injected into carotid artery after A. A, control; B, enhancement; C, recovery. Cat anesthetized with sodium pentobarbital.

of the response or paralysis. These effects are relieved by atropine (*5, 21*). Thus, atropine blocks the acetylcholine effects in exactly the same manner as in the ganglia and at the neuroeffector junctions. The unenhanced synaptic output can also be blocked by atropine, but this requires larger doses.

These indications of a cholinergic mechanism operative at these cortical and subcortical synapses of the optic system are given even more functional and physiological meaning by the results obtained with anticholinesterases. Diisopropylfluorophosphate (DFP) (*5, 21*) produces effects that are the same as those of acetylcholine and that are likewise blocked by atropine. The preservation of acetylcholine by anticholinesterases, evidenced by such effects, must presume the natural existence at these synapses of acetylcholine which acts in the same way as injected acetylcholine. To make possible the effects described, the appropriate mechanism, cholinergic in nature, must be present at the cerebral synapses studied, and the naturally available synaptic acetylcholine that activates them is attested by the anticholinesterase-induced changes. In such preparations adrenaline (*3, 5*) and nor-adrenaline (*22*), in doses within the range of physiological significance, produce pure inhibition, (Fig. 3) completing the similarity to the reciprocal relation of cholinergic and adrenergic effects pre-

[1] There are two indications that the distribution of the injection is predominantly ipsilateral on its initial passage through the brain, provided undue force is not used: (1) cortical coloration consequent to the injection of methylene blue first appears ipsilaterally, and (2) on stimulating the optic tract and recording responses from *both* optic cortices, made possible by the fact that a small proportion of optic fibers are uncrossed, the injection of acetylcholine produces effects that are greater on the side ipsilateral to the injection or contralateral to the stimulated optic tract.

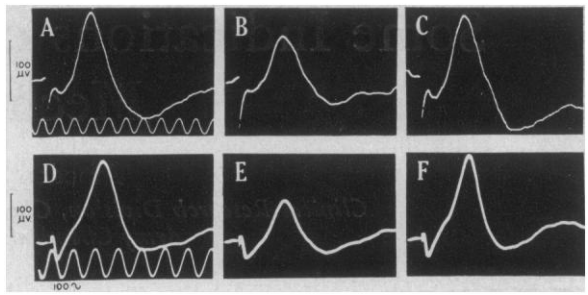

FIG. 3. Cerebral action of adrenaline and of nor-adrenaline. Potentials evoked in optic cortex by stimulation of symmetrical point in opposite cortex. Adrenaline 10 γ/kg injected into carotid artery after A. Nor-adrenaline 150 γ/kg after D. A, D-controls; B, E-inhibition; C, E-recovery. Cat anesthetized with sodium pentobarbital.

viously observed at other synapses. Both types of effects bear no dependent relation to the systemic circulatory changes that may be also induced. Moreover, the local changes in cerebral blood flow that might conceivably be expected to accompany the observed changes in blood pressure are not in the right direction to account for the excitation or the inhibition, respectively.

The evidence for the presence of an adrenergic mechanism is also supplemented by data best explained on the basis of the natural presence of adrenaline or an adrenaline-like substance at the synapses. This follows from the fact that the same inhibition is produced by other adrenaline-like substances, including amphetamine and ephedrine (*3*), which are thought to be dependent for their action on the presence of adrenaline-producing nerves (*23*). Some go as far as to say that amphetamine and ephedrine, in fact, owe their effects to a preservative action upon adrenaline (*24–26*).

The cholinergic excitatory and reciprocal adrenergic inhibitory phenomena are not peculiar to the optic system. For example, we have shown that when potentials evoked by auditory stimuli (clicks) are recorded from the auditory (temporal) cortex in the cat, the inhibitory action of adrenaline can likewise be demon-

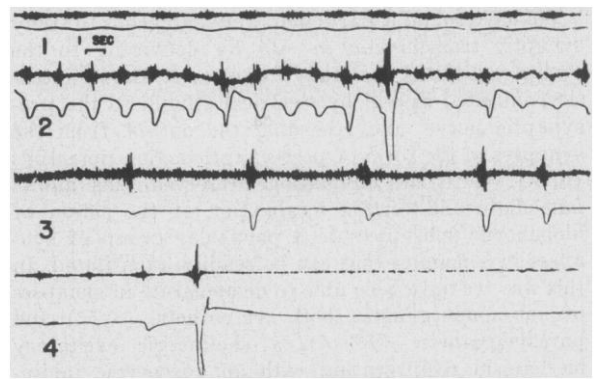

FIG. 4. Central respiratory action of anticholinesterase. Phrenic nerve potentials with associated air movement recorded at trachea. 1-control, 2-excitation, 3-beginning depression, 4-development of paralysis. DFP injected intravenously 4 mg/kg after 1; 3 mg/kg after 2; 1 mg/kg after 3. Cat anesthetized with sodium pentobarbital.

strated (*3*). In other experiments, the phrenic and intercostal nerves served as leads to the respiratory center. Thus the respiratory outflow conducted over these nerves to the muscles of respiration was recorded in the form of action potentials. Here again, central cholinergic excitation and reciprocal adrenergic inhibition were manifested as increases and decreases respectively in the trains of impulses constituting the respiratory outflow. Anticholinesterases (Fig. 4) have produced a series of changes finally terminating in paralysis that can be offset by atropine (*27*). Adrenaline (Fig. 5) and other adrenaline-like compounds, including nor-adrenaline and amphetamine, have produced inhibition (*28*).

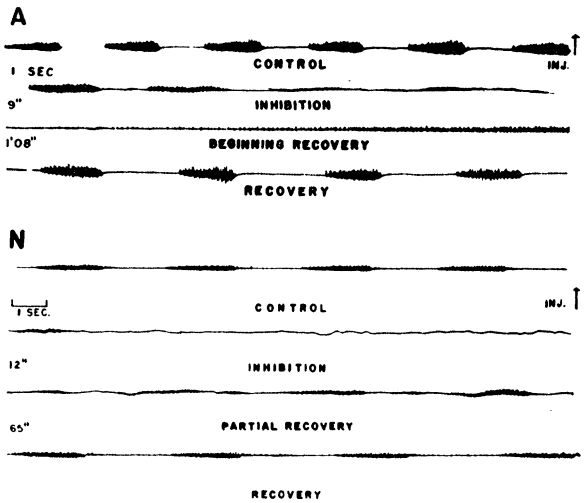

FIG. 5. Central respiratory action of adrenaline and of nor-adrenaline. Phrenic nerve potentials. Adrenaline 0.25 mg/kg injected intravenously at end of 1st line in A. Noradrenaline 0.75 mg/kg injected at end of 1st line in N. Cat anesthetized with sodium pentobarbital.

The correspondence of acetylcholine and atropine action at central (brain) and peripheral (ganglion) synapses and at other peripheral junctions, namely, neuroeffector (nerve to muscle or gland), led us to test the action on cerebral synapses of a blocking agent, which is especially characteristic for its action at junctions in skeletal muscle. D-Tubocurine is already known to block transmission in sympathetic ganglia. We have found that, in doses required to produce respiratory paralysis in the cat, it acts only at the periphery, i.e., at neuromuscular junctions, but also on the respiratory center (*27*) and on the optic cortices (*16*), in each instance producing block. The paralysis of the respiratory center indicated by cessation of the respiratory outflow over the phrenic nerve occurs early and is transient, whereas the peripheral effect is more persistent.

Another blocking agent that interests us for the light it may throw on the ubiquity of quaternary nitrogen (acetylcholine, curare, etc.) actions on junctions is tetraethylammonium (TEA), which blocks at cholinergic sites, i.e., at neuroeffector junctions and in autonomic ganglia. We have been able by our technique to quite clearly localize its blocking action at parasympathetic synapses in the ciliary ganglia and at the optic cortical synapses that we study (*22*).

It is apparent that the cerebral synapses in the terms that we have studied them resemble in detail the ganglionic synapses which, in turn, are closely analogous to the neuroeffector junctions (Fig. 6). The demonstration of cholinergic and adrenergic drug actions establishes these two specific drug-responsive mechanisms, which can both affect and effect synaptic transmission. Eliciting the same actions with preservatives or accumulators of acetylcholine and adrenaline, respectively, speaks for the natural presence of these substances at the synapse as potential activators of mechanisms that can serve humoral transmission. We are engaged in a survey of central nervous system synapses to test the extent to which the data obtained represent a more general situation characteristic of many synapses.

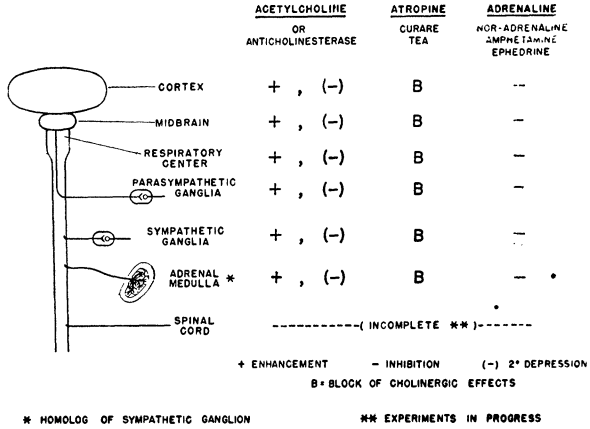

FIG. 6. Chart of cholinergic and adrenergic actions in nervous system.

Some very intriguing clinical implications present themselves. Do undoubted defatiguing agents, like amphetamine and ephedrine, exercise a mixed action, i.e., stimulating at some sites and inhibiting at others, or is the former a release phenomenon resulting from the latter? By an extension of the Hughlings Jackson concept of release phenomena, inhibition of higher controlling cortical neurons, which normally restrain activity of lower cortical neurons, could readily be conceived as manifesting itself by increased activity of the lower neurons, which would otherwise be restrained.

Pertinent to these observations are the mental phenomena that can be produced by adrenaline-like compounds—rarely with adrenaline, often with amphetamine and very conspicuously with mescaline, which also produces hallucinations that are most marked in the visual system. In cats, we have found that all three produce synaptic inhibition in the optic cortex activated in the transcallosal preparations (*29*). Accordingly, mental phenomena such as these might possibly also be looked upon as being released when synaptic inhibition interrupts pathways through

which the restraining influence of higher neurons is expressed.

The identification in the central nervous system of cholinergic and adrenergic mechanisms responsive to doses within the range of physiological interest makes it reasonable to pursue further and energetically the inquiry into the possibility of central humoral transmission along the lines that Loewi has been so instrumental in developing.

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Hydrogen Bonding as a Factor in the Ionization of Dicarboxylic Acids

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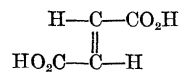
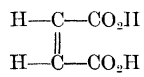
THE ratios of the first to the second ionization constant of many dicarboxylic acids show a remarkable variation with structure. Thus the ratio K_1/K_2 is 23.2 for fumaric acid (I), whereas it is 20,200 for maleic (II).

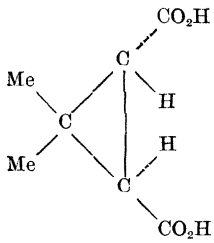


(I)

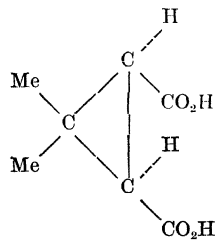


(II)

Similarly the ratio is 31.8 for *trans*-caronic (III) and 929,000 for *cis*-caronic (IV).



(III)



(IV)

These and related data are summarized in Table 1.

The deviation of the K_1/K_2 ratio of symmetrical dicarboxylic acids from the statistically expected value of four was explained by Bjerrum as due to the electrostatic effect of the negative carboxylate ion acting through the solvent medium on the remaining carboxylic hydrogen (*1*). From this assumption Bjerrum was able to calculate reasonable values for the separa-

TABLE 1

IONIZATION CONSTANTS FOR DICARBOXYLIC ACIDS IN WATER AT 25° C

Acid	10^4K_1	10^7K_2	K_1/K_2
Fumaric (*17*)	9.57	413	23.2
Maleic (*17*)	120	5.95	20,200
trans-Cyclopropanedicarboxylic (*16*)	2.2(24° C)	74(24° C)	29.7
cis-Cyclopropanedicarboxylic (*16*)	4.7	3.4(24° C)	1,380
trans-Caronic (*4*)	1.52	47.8	31.8
cis-Caronic (*4*)	45.9	0.0494	929,000
Malonic (*2*)	14.9	20.3	734
Dimethylmalonic (*2*)	6.83	8.72	783
Methylethylmalonic (*2*)	13.8	3.86	3,580
Diethylmalonic (*2*)	61.5	0.51	121,000
Succinic (*2*)	0.64	33.3	19.2
Tetramethylsuccinic (*2*)	3.19	0.52	6,130

[1] Fellow on a National Science Foundation grant at Purdue University.