References and Notes

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Positive Reinforcement Produced by Stimulating Hypothalamus with Iproniazid and Other Compounds

There is a growing body of information on the distribution of possible transmitter substances in the brain and on factors which cause temporary or long-run changes in these concentrations (*1, 2*). No technique, however, has been available to demonstrate the excitatory or inhibitory function of these chemicals in the central nervous system, nor has there been any method to demonstrate their suspected capacity to act selectively on some functional and anatomical groups without affecting others.

Pharmacotherapy in mental illness has tended to emphasize the role of epinephrine, norepinephrine, and serotonin as transmitters because these tend to be concentrated in areas of the hypothalamus and tegmentum which have proved motivational functions, and because drugs which affect mental states also affect the concentrations of these substances (*1, 2*). These three supposed transmitters are believed to be broken down in the brain by monoamine oxidases which can be inhibited by iproniazid (*1*). Thus, the latter may have its effect by augmenting the action of one or more of these three substances.

Iproniazid, in doses of 50 mg three times daily, has recently been shown to have pronounced effects in relieving severe depressions (*3*). The therapeutic effect has been tentatively related to supposedly excitatory effects of serotonin (*3*).

In our experiments (*4*), chemicals were injected into the hypothalamus of the rat in microgram amounts to test for possible rewarding effects of chemical stimulation in areas where electric stimulation is highly rewarding (*5*). Iproniazid is shown by these experiments to be a rewarding stimulant in quantities of 1 to 2 μg. The rewarding effect is specific to the chemical and to the site of injection in the brain. Iproniazid appears to share this rewarding function with epinephrine.

In these experiments, a plastic holder was screwed to the skull of each rat. From it a pair of insulated silver electrodes and a No. 26 Huber pointed hypodermic needle penetrated into the brain. This method of implantation is our own version of a technique reported by Fisher (*6*), who had based his design on an electrode developed by one of us (J. O.) (*7*).

The strategy of the series of experiments was first to canvass a range of chemical agents on the basis of a habit established by electric reward and then, after finding agents which would sustain behavior by themselves, to train a new group of animals by means of the chemical reward alone.

In the first group of experiments, electrodes and pipettes were implanted in an area of the ventral posterior hypothalamus just in front of the mammillary body. This is the center of an extensive region in which electric stimulation is rewarding (*5*). Four animals were used in these tests.

After implantation and a 14-day recovery period, animals were trained to press a lever for electric stimulation. All animals achieved rates of self-stimulation of 2000 an hour or more; the technique used has been described elsewhere (*5*). At this point they were shifted to tests of self-injection.

In self-injection studies, a polyethelene tube was used to connect a microinjector to the rat's pipette. Each lever-pressing response caused 1/700 ml of solution to be injected into the hypothalamus. The following chemicals were tested, in solutions of 1 mg per milliliter of physiological saline: acetylcholine chloride, adenosine, triphosphate disodium salt, serotonin creatinine sulfate, epinephrine hydrochloride, norepinephrine bitartrate, and Marsilid phosphate (iproniazid). Each injection contained approximately 1.4 μg of the whole compound being tested. Also, for control, physiological saline was injected alone.

The results for acetylcholine and serotonin indicate that these do not have any rewarding effect but quickly cause the animal to lose muscular tone and apparently to go to sleep. Adenosine triphosphate and noradrenaline did not have any observable effects, in the concentrations used.

Adrenaline, on the other hand, appeared to produce approach behavior; however, in the concentrations used, it caused a loss of motor coordination, and self-injection was so slow as to leave some doubt about its rewarding effects.

In the case of iproniazid, there was striking self-injection behavior. Animals injected the chemical at rates of more than 300 self-injections an hour; this is far above the chance rate obtained with saline (see Fig. 1).

In the second set of tests, electrodes and pipettes were implanted to provide

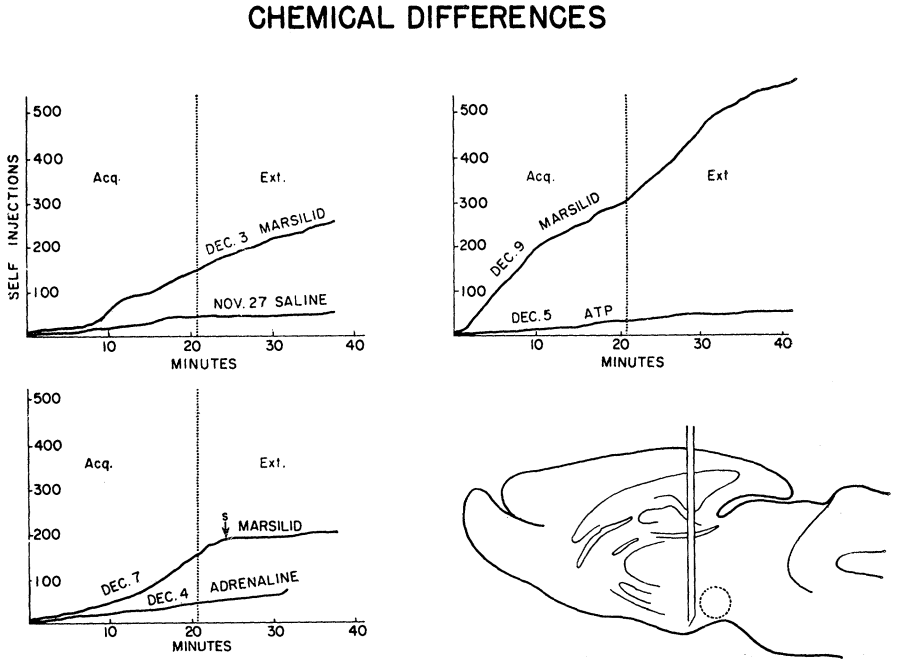

Fig. 1. Rate of response in tests with iproniazid (Marsilid) in relation to rate of response in tests with saline, adrenaline, and adenosine triphosphate (ATP). The total number of self-injections, for Marsilid, always rises to about 200 in the 20-minute acquisition period (area at left of dotted line). For saline and adenosine triphosphate, the rate does not rise above 50 (chance level). For adrenaline, the rise in rate of self-injection is slow but steady; this suggests the possibility that there are rewarding effects. Response is not rewarded by microinjections during the extinction period (area at right of dotted line). Responding tends to continue for long periods after termination of reward with Marsilid. At the point marked *S*, the animal had a seizure.

stimulation at a series of points along the floor of the hypothalamus and telencephalon. Twelve points were selected, in 12 different animals. After implantation and a 14-day recovery period, animals were trained to press a lever for chemical stimulation with iproniazid; in this case there was no prior training with electric stimulation.

Whereas all animals with ventral hypothalamic pipettes showed a better-than-chance tendency to press the bar for injection of the chemical, there was a distinct differentiation of rate of self-injection, depending on placement of the pipette. Pipettes in the posterior hypothalamus gave higher rates of self-injection than pipettes in the anterior hypothalamus. And high rates of self-injection were also obtained with pipettes in the dorsal preoptic region. These differences agree well with differences with respect to brain area in rate of self-stimulation obtained in earlier experiments with electric stimulation (*5*).

When the injector was turned off, so that bar-pressing no longer produced self-injection, animals continued to press the bar for some time (see Fig. 1), as though no change had been made. This is probably attributable to the high level of the chemical in the brain at these points and to the gradual working down of residual stores in the pipette. Extinction does eventually result from termination of the flow, after a period of about 30 to 45 minutes. Also, when the animal is shifted from an extinction period to a new self-injection period, rate of responding quickly changes from chance levels to rates of about 300 an hour. Thus, it is the chemical reward which sustains the behavior.

Injection of serotonin, by itself or immediately after injections of iproniazid, caused the animals to lie down. An animal lying down after injection of serotonin could be brought back to its feet by epinephrine. This, taken together with the tendency of some animals to press the bar for epinephrine, suggests that the exciting and rewarding effects of iproniazid are connected more with epinephrine than with serotonin.

From the experiments on self-injection, three main results have been gained. Substantively, we have learned that iproniazid is an excitant of reward functions in this motivational system of the hypothalamus, and that quite probably it has this excitatory function in common with epinephrine. Methodologically, we have validated a technique of self-injection which can now be used to resolve further the problem of the excitatory and inhibitory chemistry of the motivational systems. Finally, from the clinical point of view, we have been exceptionally fortunate in having our method select the pharmacological agent which has recently given best results in alleviating depressions, for this indicates that it is a method for locating antidepressants.

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A New Phospholipid, Malignolipin, in Human Malignant Tumors

We report here the discovery of a new phospholipid—malignolipin—containing spermine, found specifically in malignant tumors, but never in normal tissues. Laborious efforts of many of our predecessors have been concentrated on the problem of finding a substance which exists only in malignant tumors and never in normal tissues, but such efforts have been unsuccessful up to the present.

In examining the affinity of various cell components for porphyrin (*1*), we noticed the very marked affinity of extracellular small bodies in cancer tissues for protoporphyrin III (*2*), and we also ascertained that the substance in normal tissues which has an affinity for protoporphyrin III, and which occurs in mitochondria and myelin sheaths, is sphingomyelin (*3*). Investigation of the chemical nature of the extracellular small bodies in cancer tissues with the marked affinity for protoporphyrin III has led to the discovery of a new phospholipid.

Sphingomyelin can be extracted by means of boiling 95-percent ethanol; the substance other than sphingomyelin with affinity for protoporphyrin III is isolated from freshly excised human malignant tumors (a seminoma, a stomach cancer, a colon cancer, a uterine cancer, a breast cancer, and Hodgkin's malignant granuloma were used in the studies reported here) by fractionation with organic solvents, as follows. A freshly excised malignant tumor is extracted with 9 volumes of boiling ethanol and filtered while hot. The filtrate is left at 0°C overnight, and the supernatant is evaporated to dryness in a vacuum, then extracted with absolute ethanol. The supernatant is added, with 2 volumes of acetone, and the solution is left at 0°C overnight. The precipitate is washed with acetone-ethanol (2:1) and then with acetone; then it is dried in a vacuum and dissolved in ether. The supernatant is added, with 2 volumes of acetone, and the solution is left at 0°C overnight. The precipitate is washed with acetone, dried in a vacuum, and dissolved in absolute ethanol. The supernatant is added, with 2 volumes of acetone, and the solution is left at 0°C overnight. Such precipitation with acetone from ether and ethanol solution is repeated till the final precipitate contains no trace of the substance that has no affinity for porphyrin (*3*). The precipitate is washed with acetone-ethanol (2:1), then with acetone; then it is dried in a vacuum and dissolved in chloroform. The supernatant is added, with 2 volumes of acetone, and the solution is left at 0°C overnight. The precipitate is washed with acetone, freed from acetone, and crystallized from chloroform. The substance with the affinity for protoporphyrin III was obtained thus, in pure state, as small hexagonal, snow-crystal-like crystals, from every one of the aforementioned malignant tumors.

This compound is very hygroscopic, is strongly basic, and is readily soluble in water, ethanol, ether, petroleum ether, and chloroform but is insoluble in acetone. In every instance, the compound obtained from the aforementioned tumors was found to contain nitrogen and phosphorus in the ratio of 5 to 1 and, quite unlike all other phospholipids that have ever been reported, to show only one spot; this spot can be revealed by Ninhydrin, as well as by Dragendorf's reagent, on the paper chromatogram developed with *n*-butanol, butanol–acetic-acid–water (4:1:5), or ethanol-water (8:1). Moreover, the compound was found not to be contaminated with other substances (the biuret test, Ehrlich's aldehyde test, Molisch's test, Bial's test, Pettenkofer's test, Feulgen's test, and the Liebermann-Burchard test were all negative).

When this lipid is left in 0.3*N* HCl at 18°C for 3 hours, the opalescent solution becomes quite transparent, with some oily droplets at the surface, the whole amount of phosphorus contained in the original lipid can be recovered as free phosphoric acid, and no trace of acetone-insoluble original lipid can be obtained from its ether extract—that is to say, the lipid is completely hydrolyzed.

The ether-soluble part of the hydrolyzate contains neither phosphorus nor nitrogen, and the following tests have all been negative: Bial's test, Molisch's test, Ninhydrin test, sodium fluorescein test, Feulgen's test, and the Liebermann-Burchard test. It has been ascertained that the ether-soluble part of the hydrol-