The Journal of Asthma Research Vol. 16, No. 2, January 1979

The Intracranial Injection of Drugs in Goldfish. I: Hallucinogens and Their Antagonism to Smooth Muscle Activity*

H. A. ABRAMSON, M.D., H. H. GETTNER, M.A., P. A. CARONE, M.D., A. ROLO, M.D., AND L. KRINSKY, PH.D.

For many years, this laboratory has been studying psychotomimetic and other drugs, assaying drug response by observing the surfacing reaction in goldfish and Siamese fighting fish.[1, 2, 3, 4] In the first experiments, the drug to be tested was dissolved in the liquid in which the fish swam. In these experiments, a constant reservoir of the drug was available to be ingested or absorbed by the fish gills. In further studies to find a method for studying possible blocking effects of test drugs on the LSD reaction, drugs were injected into the body cavity, while LSD was in the outside liquid. In this way, potential interactions between the injected drug and the drug in the outside liquid were decreased.

In this report we believe that a more sensitive method of testing drugs has been developed by injecting drugs intracranially into goldfish. By injecting directly into the brain area, the procedure becomes more efficient. For a drug like mescaline, such large quantities are needed for testing that toxic reactions occur; however, by using the intracranial technique we were able to obtain typical surfacing reaction to mescaline in keeping with its action on man.

Method

A goldfish weighing about 1½ to 3 grams is lightly held between the thumb and index finger. Using a 30-gauge needle and a syringe graduated to 0.1 ml, the fish is injected intracranially in the mid-line tangent to the rim of the eye-socket with .01 ml of test solution. The fish is then placed in 1 liter of water and observed for possible brain injury. If there is brain injury, which is easily noted by the erratic swimming pattern, the fish is removed from the beaker. After ten fish have been successfully injected, they are transferred to a liter cylinder. Air is bubbled through the liquid as described previously. At ten-minute intervals, the air is turned off and the fish are observed for surfacing reaction characteristic of their response to many hallucinogens.

Experimental

In all the curves to be discussed, the ordinate represents the average percent of goldfish surfacing. Figure 1 compares the effect of 0.2 mcg, 0.5 mcg and 1.0 mcg of LSD-25 (d-lysergic acid diethylamide) injected intracranially. Note the rapid rise of the fish to the surface, where they remain for the period of observation (60 minutes). However, the reaction differs markedly from that

* From the South Oaks Foundation, Amityville, New York 11701

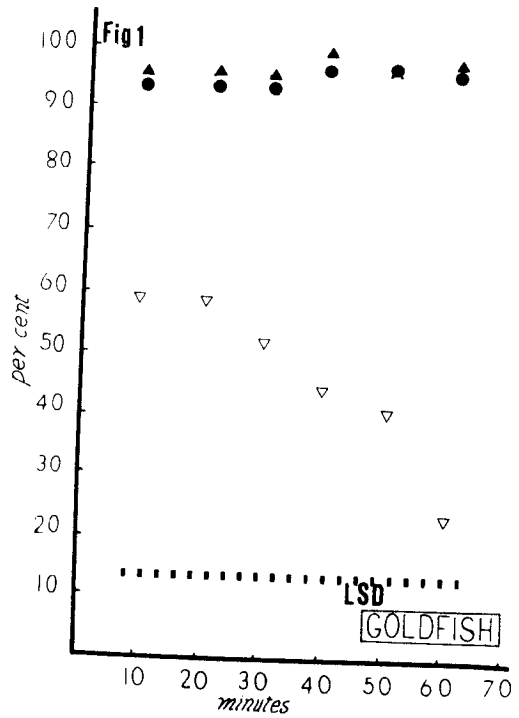

FIG. 1.—The ordinate indicates the average per cent of goldfish surfacing following intracranial injection of d-lysergic acid diethylamide (LSD-25). ▽ 0.2 mcg, N = 30; ● 0.5 mcg, N = 30; ▲ 1.0 mcg, N = 40. ▬: control.

observed when the fish are suspended in a solution of LSD-25. This difference is illustrated in Figure 1A. The reservoir of LSD-25 in the outside liquid results in the maintenance of the fish at the surface. When injected intracranially, the LSD seems to be rapidly metabolized when the quantity injected is 0.2 mcg.

Figure 2 represents data obtained with ALD-52 (d-2-acetyl lysergic acid diethylamide). In man, ALD-52 is almost as effective as LSD-25. However, Figure 2 indicates either that our sample had deteriorated or that the diffusion of ALD-52 was slower than that of LSD-25, the acetyl group probably changing the transport across the blood brain barrier.

As in man, UML-491 (1-methyl d-lysergic acid butanolamide) is much less active. LSD is approximately 200 times more effective in producing psychological effects in man. In Figure 3, 40 mcg of UML-491 was not as effective as 0.5 mcg of LSD. In other words, the ratio of the effect in fish and in man is about the same for the intracranial injection method in fish.

Is the surfacing reaction restricted to LSD, its congeners and derivatives? Figure 4 shows that as little as 10 mcg of a comparatively simple drug, 5-methoxy dimethyl tryptamine, is quite effective in producing the surfacing reaction typical of LSD. This is consistent with the wide range of drugs considered to be hallucinogenic in man.

The approximate order of activity which produces 50 percent of surfacing (SR_{50}) in 30 minutes, on the basis of the figures, is:

LSD	0.2 mcg
ALD	0.2 mcg
UML	5.0 mcg
5-MEO-DMT	5.0 mcg

Discussion

It is anticipated that the drugs of special interest to the allergist will be part of this study. As a matter of fact, UML (Sansert) is the best drug known for the prevention of certain headaches, many of which may be of allergic origin. The antiserotonin activity of UML is among the highest of certain LSD congeners and derivatives of LSD.

What have psychotomimetic drugs to do with allergic mechanisms? For many years the dominant notion in the therapy of the allergic state was based on neutralizing the effects of histamine. There were many contradictions and paradoxes to this view, especially the failure of the antihistamines in the treatment of asthma. More recently serotonin (5-hydroxytryptamine) has been

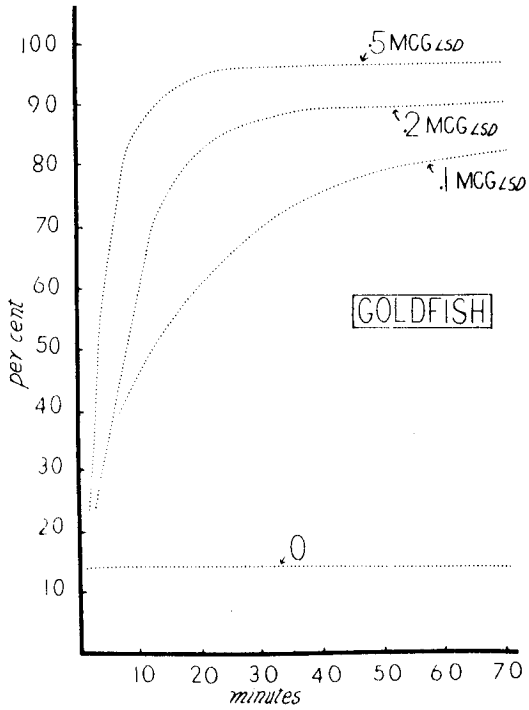

FIG. 1A.—The curves illustrate the difference between the action of LSD-25 in the outside liquid and the response when injected intracranially as in Figure 1. As in Figure 1 the ordinate represents the average per cent of fish surfacing.

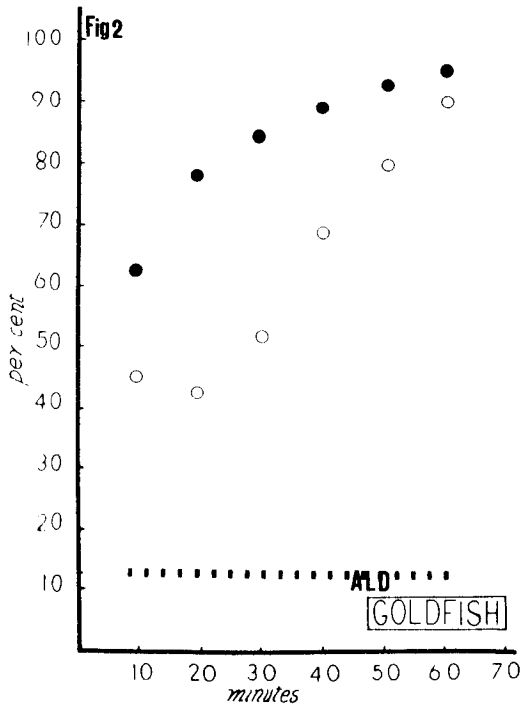

FIG. 2.—The ordinate indicates the average per cent of goldfish surfacing following intracranial injection of 1-acetyl d-lysergic acid diethylamide (ALD-52). ○ 0.2 ALD, N = 30; ● 0.5 ALD, N = 40. ▬: control.

brought into the picture not only as an agent possibly connected with emotional disturbances but also as a mediator in the symptoms of allergic phenomena. Thus Waalkes[5] and his coworkers have shown that serotonin 5-HT is released *in vitro* from rabbit platelets following antigen-antibody reactions and *in vivo* during anaphylaxis because clumped platelets and white cells collect in pulmonary vessels during the anaphylactic response. In the rabbit, the quantity of both serotonin and histamine in the lung is increased.

O'Brien, Hughes and Newberne[6] point out that serotonin is known to be involved in the anaphylactoid reaction elicited by dextran and egg white. The same authors showed that d-lysergic acid diethylamide (LSD-25) blocked the course of experimental allergic encephalitis. Fifty mcg triweekly reduced the incidence of paralysis, the mortality rate and the severity of the histopathologic lesions in guinea pigs with allergic encephalitis.

5-HT was found to cause contractions of the uterus, duodenum, and colon of rats; the uterus, duodenum, and jejunum of rabbits; and the uterus, duodenum, jejunum, and ileum of guinea-pigs. It caused vasoconstriction in the perfused ears of rabbits. Since smooth muscle is of the utmost importance in allergic phenomena, the early work of Gaddum[7] and his coworkers will be briefly reviewed here.

Of special importance are derivatives of lysergic acid because tryptamine forms part of the molecule of both lysergic acid and serotonin. The most active and specific of these derivatives, Gaddum found, was lysergic acid diethylamide, or LSD. Various derivatives of lysergic acid were compared for their effects on the rat's uterus as antagonists of serotonin, and Gaddum and Hameed report the following,[7] in descending order of potencies: lysergic acid diethylamide, dihydroergotamine, dihydroergocornine, and dihydroergokryptine. Tests on the rabbit's ear disclosed that serotonin was more depressed than adrenaline. Serotonin in one experiment had more vasoconstrictor action than adrenaline in the rabbit's ear. LSD was then perfused, and this abolished the constrictor effect of serotonin even when the dose of serotonin was rather high.

One of the conclusions is that the effects of various serotonin antagonists can be explained on the theory that there are two types of tryptamine receptors. One type is present in the smooth muscle of the uterus and ear and is specifically inhibited by low concentrations of LSD. The second type is present in the nervous tissues in the ileum and is not inhibited by LSD. Fleisch, Kent and Cooper[8] in a volume edited by Austin and Lichtenstein point out that serotonin produced contraction of rat and guinea-pig tracheal musculature. This was blocked by LSD. Although tracheal musculature was used, this

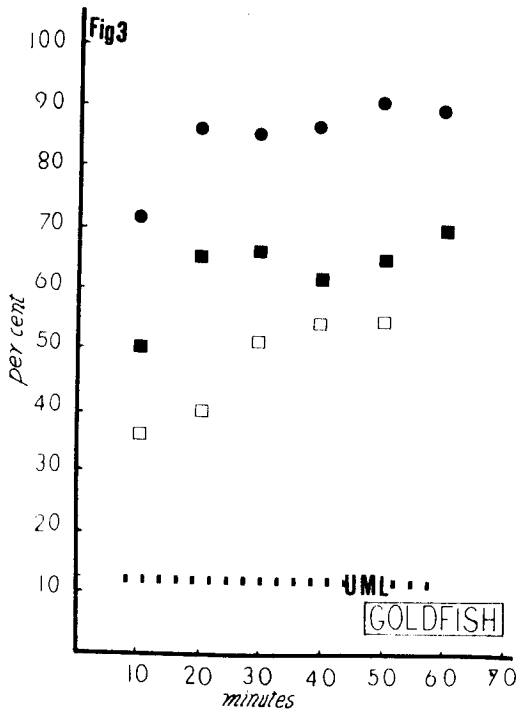

FIG. 3.—The ordinate indicates the average per cent of goldfish surfacing following intracranial injection of 1-methyl-d-lysergic acid butanolamide (UML-491). ● 40 mcg, N = 30; ■ 20 mcg, N = 60, □ 5 mcg, N = 40. ▬: control.

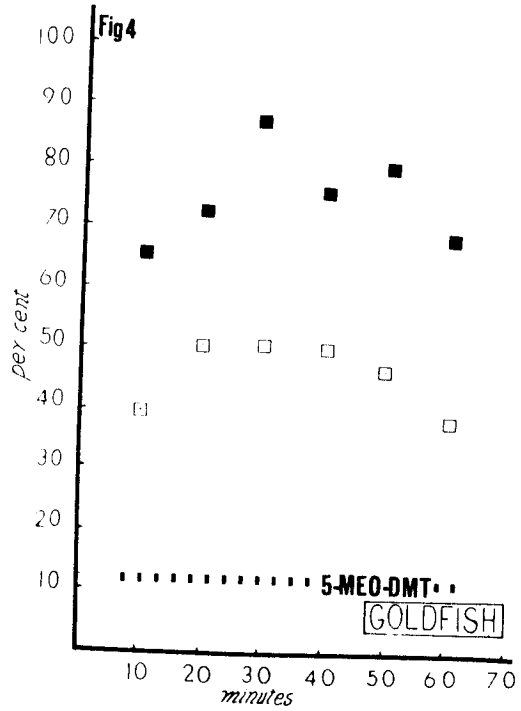

FIG. 4.—The ordinate indicates the average per cent of goldfish surfacing following intracranial injection of 5-methoxy dimethyl tryptamine (5-MEO-DMT). □ 5 mcg, N = 30; ■ 10 mcg, N = 60. ▬: control.

is not as ideal as experiments which could be performed either on the whole lung itself or on the smooth muscles of the smaller bronchioles.

The pharmacology of sheep tracho-bronchial muscle was investigated by Eyre.[9] Serotonin and acetylcholine contracted the musculature in all parts of the respiratory tract, and isoproteronol relaxed it. Histamine contracted the tracheal and major bronchial muscles but relaxed the lesser bronchi and bronchioles. The authors maintain that within the bounds of species differences, the respiratory tree contains as diverse a group of pharmacological receptors as any organ in the body.

According to Cerletti and Doepfner,[10] the quantitative and qualitative characterstics of the 5-HT antagonism of a large number of natural and semi-synthetic "ergot derivatives" have been studied on the isolated rat uterus. Within a group of about twenty amide derivatives of lysergic acid, more or less closely related to LSD-25 from a chemical point of view, none reached the degree of activity and specificity of LSD. Substances with antiserotonin activities higher than LSD were found within a group of LSD derivatives with different substitutions on the ring structure of lysergic acid. The most potent compound studied was the 1-methyl-2-bromo lysergic acid diethylamide.

Summary

A simplified method of studying the surfacing reaction of goldfish to hallucinogens is described. Goldfish weighing up to three grams are injected intracranially. Employing this method, d-lysergic acid diethylamide (LSD-25), d-2-acetyl lysergic acid diethylamide (ALD-52), 1-methyl d-lysergic acid butanolamide (UML-491), and 5-methoxy dimethyl tryptamine (5-MEO-DMT) were found to be as pharmacologically active as previously noted in fish and in man. The relationship of these drugs to their anti-serotonin activity is of particular interest to the allergist because of the way in which the congeners and derivatives of LSD block the action of serotonin on smooth muscle.

References

1. Abramson, H. A., and Evans, L. T. Lysergic Acid Diethylamide (LSD-25): Psychobiological Effects on Siamese Fighting Fish, *Science 120*:990, 1954.
2. Weiss, B., Abramson, H. A., and Baron, M. O. Lysergic Acid Diethylamide (LSD-25): XXXVII. Effect of Potassium Cyanide and Other Oxidase and Respiratory Inhibitors on the Siamese Fighting Fish, *J.A.M.A. Arch. of Neurology and Psychiatry 181*:1136, 1958.
3. Abramson, H. A., Gettner, H. H., Rolo, A., and Dean, G. Lysergic Acid Diethylamide (LSD-25): XXXIV. Comparison with Effect of Psilocybin on the Siamese Fighting Fish, *J. Psychol. 56*:363–374, 1963.
4. Gettner, H. H., Carone, P. A., and Abramson, H. A. Lysergic Acid Diethylamide (LSD-25): Effect of pH on Transport Across Gill Membrane, *J. Psychol. 84*:111, 1973.
5. Waalkes: See AAS, Kjell. *The Biochemical and Immunological Bases of Bronchial Asthma*, pp. 13, 130–131, C. C. Thomas, Springfield, Ill., 1972.
6. O'Brien, D. J., Hughes, F.W., and Newberne, J. Influence of Lysergic Acid Diethylamide on Experimental Allergic Encephalomyelitis (27832), *Soc. for Exp. Biol. and Med. 111*:490, 1962.
7. Gaddum, J. H., and Hameed, K. A. Drugs Which Antagonize 5-Hydroxytryptamine, *Brit. J. Pharmacol. 9*:240, 1954.
8. Fleisch, J. A., Kent, K. M., and Cooper, T. *Asthma*, edited by Austen, K. F., and Lichtenstein, L. M., Academic Press, New York, 1973.
9. Eyre, P. *Brit. J. Pharmacol. 36*:409, 1969. Cited by Fleisch, Kent and Cooper.
10. Cerletti, A., and Doepfner, W. Comparative Study on the Serotonin Antagonism of Amide Derivatives of Lysergic Acid and of Ergot Alkaloids, *J. Pharmacol. and Exp. Therap. 122*: 124, 1958.

Further important references:

Borges, F. J., and Bessman, F. P. Serotonin (Editorial), *Ann. Int. Med. 46*:425, 1957.

Weissbach, H., Waalkes, T. P., and Udenfriend, S. Presence of Serotonin in the Lung and its Implication in the Anaphylactic Reaction, *Science 125*:235, 1957.

Fink, A. M. Anaphylaxis in the Mouse: Possible Relation of the Schultz-Dale Reaction to Serotonin Release, *Proc. Soc. Exp. Biol. Med. 92*:673, 1956.

The well-indexed volume edited by Sankar, D. V., Siva, *LSD—A Total Study*, PJD Publications, Westbury, N. Y. 11590, provides a source of recent research from the neurochemical, physiological and stereochemical points of view.