LYSERGIC ACID DIETHYLAMIDE (LSD 25): XXXX. EFFECT OF pH ON TRANSPORT OF METHYSERGIDE AND LSD 25 ACROSS GILL MEMBRANE* [1]

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SUMMARY

The effect of pH on the transport of lysergic acid diethylamide (LSD 25) and of methysergide has been studied in both Siamese fighting fish and goldfish. Two methods have been employed to study the transport of these drugs. In one method, the fish were immersed in a solution of the drug, thus providing an essentially unlimited supply of the drug in the outside liquid during the course of the experiment. In the second method, the fish were dipped for a much shorter period and then observed after the excess drug had been washed off. If has been found that the pH of the outside liquid, in both methods, is a major factor controlling the transport of LSD 25 and methysergide across the gills. Between pH 5.0 and pH 5.7, the surfacing reaction of the fish is diminished, with maximum blocking occurring at pH 5.0, the blocking effect diminishing to zero at pH 5.8. The data are discussed in connection with the permeability of the blood brain barrier of the nervous system to psychotomimetic drugs.

A. INTRODUCTION

In preliminary experiments (2), histidine, glutamic, and dl-aspartic acids (at pH 6.0) seemed to partially block the transfer of LSD 25 across the gill of the Siamese fighting fish as determined by the surfacing reaction. For a randomly selected group of other simple amino acids, the pH had to be lowered to 5.0-5.6 to obtain the blocking effect. The study of the effect of pH of the liquid bathing the gills on gill permeability therefore seemed pertinent. For this latter study we used goldfish rather than Siamese fighting fish. LSD 25 and the butanolamide of d-lysergic acid (methysergide) were employed in these studies.

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[1] The authors' thanks are due Sandoz Pharmaceuticals for generous donations of methysergide used in these experiments.

The word transport here is used to indicate movement of compounds studied through a membrane without reference to chemical or physical processes occurring within the membrane itself.

B. Methods

Two methods were employed to study the effect of pH on the passage of LSD 25 and methysergide through the gills of goldfish. In the first method, 10 goldfish, one to one-and one half inches in length are placed into liter graduate cylinders (height = 35 cm) containing one liter of test solution, thus providing a large and fairly constant quantity of drug throughout the experiment. A slow, steady stream of air is bubbled through each cylinder containing the goldfish. Similar techniques were used previously (2) for Siamese fighting fish, but without the air stream. For each reading of the number of fish surfacing when air bubbling is used, the air is turned off to avoid having the fish brought to the surface by the stream of air bubbles. The number of nose-up, tail-down fish surfacing is observed every 10 minutes.

In the second method, five goldfish, one to one-and-one half inches in length, are dipped into 100 ml of the test solution for five minutes and then washed successively for 10 seconds in four beakers, each containing 100 ml of distilled water. The fish are then placed into liter cylinders containing one liter of distilled water (no drug) with air bubbling through. The number of fish surfacing in the nose-up, tail-down position is noted at 10-minute intervals, as in the first method. From a methodological viewpoint, the dipping method corresponds to a rapid intravenous infusion of the drug, the transfer occurring across the gill membranes.

C. Results

In Figure 1 are the results of experiments ($n = 290$) reported previously on Siamese fighting fish after immersion in solutions of LSD 25 for different initial concentrations (2). The solid black lines in Figure 1 show the standard surfacing reaction when the fish are constantly immersed in solutions of LSD 25. Compare the surfacing reaction when the fish are briefly dipped into solutions of LSD 25 for 30 seconds. The concentrations of LSD 25 varied from 5 to 50 mcg/ml. The die-away curves are very different from the standard solid line persistent immersion curves. The die-away curves indicate in our opinion that the LSD 25 diffused primarily through the gills when the fish were dipped into LSD 25. The LSD 25 is probably destroyed by metabolic processes, rather than excreted. Dipping is probably equivalent

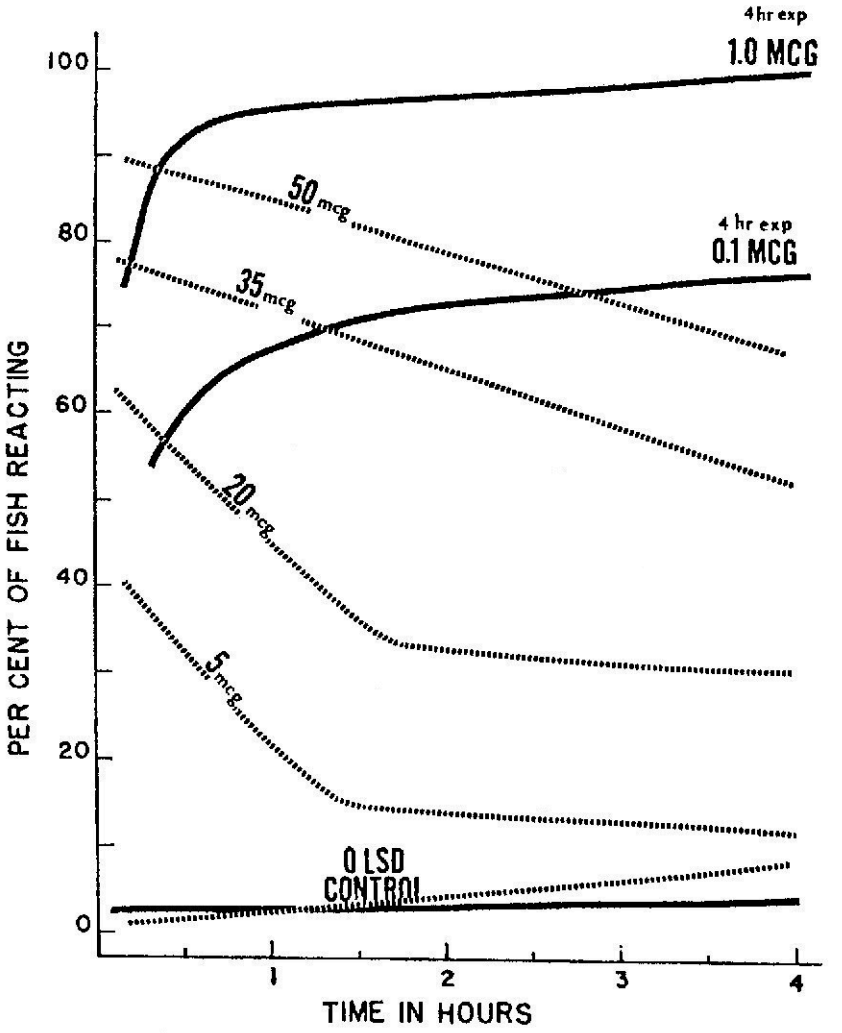

FIGURE 1

EFFECT OF DIPPING FISH INTO LSD SOLUTIONS OF VARYING CONCENTRATIONS (TIME—30 SECONDS)

The solid black lines show the surfacing reaction of Siamese fighting fish when immersed in solutions of LSD 25. The dotted lines represent the surfacing reaction when fish are dipped for 30 seconds into solutions of LSD 25 varying in concentration from 5 to 50 mcg/ml.

to an injection. Die-away curves are obtained both after injection and dipping. Similar die-away curves were obtained with goldfish by dipping for 30 seconds into solutions of LSD 25 or higher concentrations of methysergide. In connection with our belief that transport is primarily through the gills, the Information Bulletin of Sandoz, Ltd., Basle, Switzerland, points out that when methane sulfonate of ethyl m-aminobenzoate is used as an anesthetic, the solution is active if it is sprayed on the gills of large fish; e.g., sharks or rays. Given orally to mammals, the anesthetic produces no effects.

1. *The Effect of Goldfish on the pH of the Medium*

HCl solution was added to distilled water to bring the pH to 5.0. Ten goldfish swimming freely, as indicated under Methods, within 10 minutes changed the pH to 5.4-5.5. At the end of one hour, the pH had risen to 5.8 ($n = 30$). Similarly, in experiments using LSD 25 or methysergide, when the pH was 5.0 at the start of the experiment in an unbuffered solution, it rapidly rose, finally reaching pH 5.7 at the end of one hour. This is probably due to secretion of basic material. When the pH initially was 6.0, there was little if any pH change at the end of one hour. This rise in pH complicated the experimental procedure somewhat, since buffers were required. With .01 M sodium phosphate buffer, no appreciable pH change occurred during an experiment of one to two hours. The effect of LSD 25 and methysergide on the surfacing reaction of the goldfish was then readily studied. The presence of LSD 25 or methysergide in our system does not materially affect the rise in pH.

2. *Is pH 5.0 a Suitable Medium for Goldfish?*

According to authorities (1) and our own experience, water at pH 5.0 adjusted with HCl or with phosphate buffer and HCl does not damage the fish. Fish kept in solutions of pH 5.0 to pH 7.0 for hours do not show gross evidence of damage. To demonstrate the rapid and reversible change in transport of LSD 25 as measured by the surfacing reaction as a function of pH, see Figure 2. In Figure 2, the solid black curve at the bottom demonstrates the slow transport (surfacing reaction) of LSD 25 across the gill membrane when base secreted by the fish is neutralized by small additions of HCl to maintain the pH near 5.0. At the end of one hour, the pH was rapidly changed to 5.7 by addition of NaOH. Note in Figure 2 the rapid surfacing of the fish corresponding to the surfacing produced by the remaining reservoir of LSD 25 in the outside liquid. The concentration of

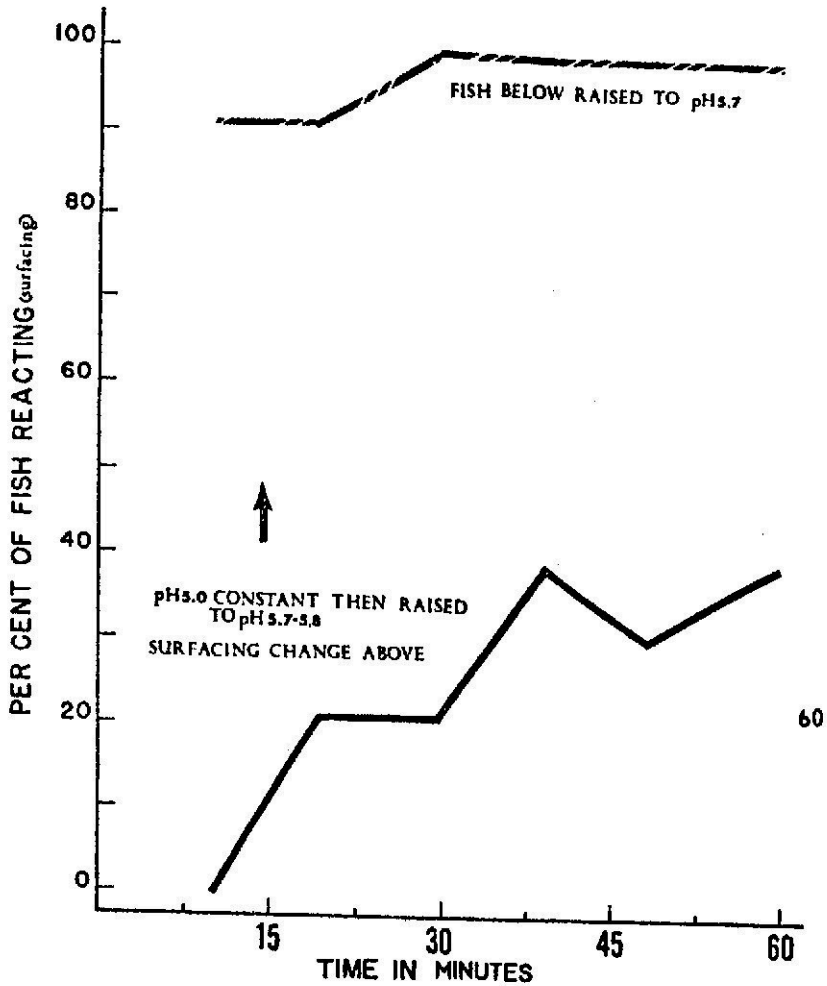

FIGURE 2

EFFECT OF SUDDEN RISE IN pH ON SURFACING OF FISH

The solid black curve at the bottom demonstrates the slow transport of LSD 25 across the gill membrane when base secreted by the fish is neutralized by small additions of HCl to maintain the pH near 5.0. At the end of one hour the pH was rapidly changed to about 5.7 by addition of NaOH. Note the rapid surfacing of the fish corresponding to the surfacing produced by the remaining reservoir of LSD 25 in the outside liquid.

LSD 25 at pH 5.0 was apparently not materially affected during the experiment.

3. *The Cutoff pH of Blocking in Simple Systems Using HCl*

In order to approximate the pH at which there seemed to be little effect of pH in simple systems, HCl was added initially to the LSD 25 or methysergide solution to adjust to the desired pH, and observations were made for one hour. With HCl between pH 5.5 and 5.8, it appeared that the effect of pH becomes negligible at approximately pH 5.8 for .5 mcg/ml of LSD 25. But below pH 5.7-5.8, the transport of LSD 25 across the gill membrane is definitely decreased. The final surfacing reaction, in the absence of buffers, however, is about the same after one hour because of two factors: increase of pH and a large reservoir of LSD 25.

4. *Comparison of LSD 25 and Methysergide by the Dipping Method*

With use of the dipping method, the surfacing of goldfish dipped for five minutes in .01 M sodium phosphate buffer containing 3.5 mcg LSD 25/ml at pH 5.0 was compared with those dipped into a similar LSD 25 buffer solution at pH 6.0. Since almost 100% of those dipped at pH 6.0 surfaced rapidly, the blocking reaction is calculated from the number of fish that do not surface at pH 5.0. That is, the percent of fish that do not surface is the percent of blocking. The data at pH 6.0 should be considered as 0% of blocking and are therefore not included in Table 1. Similar experiments were run with methysergide at a concentration of 50 mcg/ml. The data in Table

TABLE 1
BLOCKING OF SURFACING REACTION OF GOLDFISH AT pH 5.0[a]

Goldfish *N*	Concentration	Dipping time (minutes)	% blocking at time in minutes 10	20	30	40	50	60
			LSD 25 solution					
40	3.5 mcg/ml	5	35	42	56	64	60	67
10	1.75 mcg/ml	5	44	56	57	71	80	100
10	3.5 mcg/ml	2.5	60	50	66	37	25	37
			Methysergide solution					
35	.5 mg/ml	5	26	31	37	40	40	46
10	.25 mg/ml	5	20	50	83	80	100	75
10	.5 mg/ml	2.5	38	29	43	29	20	34

[a] Buffer: .01 M sodium phosphate solution. The fish were dipped into 100 ml of (*a*) LSD 25 solution in the first set of tests and (*b*) methysergide solution in the second set of tests; and then they were washed four times successively for 10-second periods in 100 ml of distilled water.

1 show unequivocally that blocking of the surfacing reaction occurs at pH 5.0 by use of the second method, that of dipping for a brief period.

Since the amount of LSD 25 and methysergide that goes through the gills while in contact with LSD 25 or methysergide as determined by the surfacing reaction is less at pH 5.0 than pH 6.0, the effects at pH 5.0 are like those of a lower concentration of the drug at pH 6.0.

D. Discussion

Our interest in the transfer of LSD 25 and methysergide across the gill membrane was originally engendered by earlier observations that, under certain conditions, beef brain and other tissue extracts blocked this transfer. Later observations indicated that the pH of the medium bathing the fish, and therefore the gills, markedly affected the percentage of fish surfacing when LSD 25 or methysergide was in the medium. We assume here that the effect of pH reported is primarily due to the effect on the gill transport system, and not due to the ionization or charge of the drugs sharply changing between pH 5.0 and 5.7, or to the overall effect of these pH changes on the skin reflexly influencing the gas content of the swim bladder.[2] The fact that the pH of the medium may influence absorption through living membrane is well borne out by data of Travell (5) on the stomach of the dog and cat, with use of alkaloids. In animals with ligated cardia and pylorus, alkaloids are not absorbed to any extent from the stomach when the reaction of the gastric juice is strongly acid. If the gastric juice is rendered alkaline, however, alkaloids are rapidly absorbed from the ligated stomach. It is of interest that absorption slows at about pH 5.0 and is fairly rapid at pH 6.0.

Experiments on the transport of LSD 25 and its congeners and derivatives are not without clinical interest. The permeability of the blood brain barrier to LSD 25 is low. It is of importance to determine all those conditions that lead to the absorption of drugs from the blood stream into the central nervous system. Without data on living tissues, theoretical extrapolations for the psychotomimetic drugs are rarely valid. Another factor to be considered is that the pH at the surface of a tissue is in all likelihood different from the pH of the medium itself. In the case of the gill, ammonia according to Smith (4), is excreted. When absorption occurs, the living membrane is a dynamic system, and it is not surprising that the value of the pH of the medium influences the dynamics at the cell's surfaces.

[2] We do not have available the dissociation curves of these compounds between pH 5.0 and 5.7. These will be obtained, if possible, and considered, if necessary.

In the case of the gill, ammonia is excreted, according to Smith. However, in our opinion, it is difficult to visualize ammonium hydroxide being responsible for the change. We feel it is more likely that the gill would act very much like the pancreas and that the increase in pH of the outside liquid from pH 5.0 to pH 5.8 is due to the excretion of bicarbonate. This is discussed at length by Lehniger (3).

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