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Activity of Local Anesthetic Agents in Goldfish

STUART FELDMAN ˣ, MARY DeFRANCISCO, and PETER J. CASCELLA

Abstract □ The activity of procaine hydrochloride, lidocaine hydrochloride, tetracaine hydrochloride, and dibucaine hydrochloride in producing overturn in goldfish was measured in pH 8.0 buffer. Calculation of the apparent minimum effective concentration of local anesthetic necessary to result in overturn of the goldfish showed that the activity of these agents increased in the following order: procaine hydrochloride < lidocaine hydrochloride < tetracaine hydrochloride < dibucaine hydrochloride. The effect of these agents on goldfish can be correlated with previous work on the minimum concentration necessary to block conduction in isolated nerve and muscle fibers. The activity of the local anesthetic agents could be explained, in part, by the relationship between the chloroform–pH 8 buffer partition coefficient and the minimum effective concentration in goldfish. Experimental results indicate that the unionized drug molecule is responsible for the observed effects.

Keyphrases □ Anesthetics, local—activity of procaine hydrochloride, lidocaine hydrochloride, tetracaine hydrochloride, and dibucaine hydrochloride in producing goldfish overturn, structure–activity relationships □ Structure–activity relationships—local anesthetics, goldfish overturn □ Goldfish—model for determining structure–activity relationships of local anesthetics

Recent reports (1–3) indicated that goldfish may serve as a biological test model in the assessment of structure–activity relationships of drugs. The goldfish was an adequate test system for discerning structure–toxicity relationships of substituted phenothiazine derivatives (1), and the goldfish was used as a model to study the effects of alkyl chain length on the alkyl *p*-aminobenzoate-induced narcosis in the fish (2). The use of this simple and inexpensive biological test system to study the correlation between pharmacological and toxicological activity with physical–chemical properties has many advantages (1). The present report concerns preliminary findings as to the relationships that may exist between structure and activity of local anesthetic agents in goldfish.

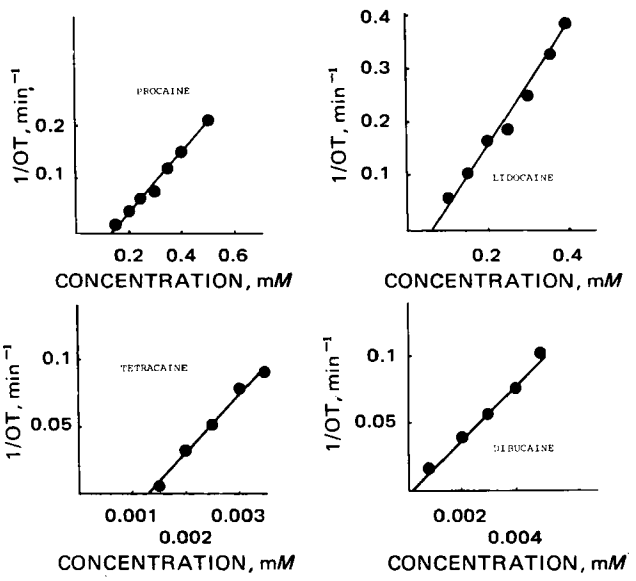

Figure 1—*Plot of the reciprocal of overturn time of local anesthetics in goldfish* versus *concentration of local anesthetic agent.*

EXPERIMENTAL

Goldfish, common variety (*Carassius auratus*), weighing 3–4 g were purchased locally. The overturn time (4) of individual goldfish in 100 ml of various concentrations of procaine hydrochloride[1], lidocaine hydrochloride[2], tetracaine hydrochloride[1], and dibucaine

[1] Amend Drug and Chemical Co., Livingston, N.J.

[2] Astra Pharmaceuticals, Worcester, Mass.

Table I—Average Overturn Times Produced by Various Concentrations of Local Anesthetic Agents in pH 8.0 Buffer

Agent	Concentration, m*M*	Number of Fish	Mean Overturn Time, min	Standard Deviation	Mean Reciprocal of Overturn Time, min^{-1}
Procaine hydrochloride	1.5×10^{-1}	5	63.1	15.5	0.016
	2.0×10^{-1}	5	24.2	8.00	0.041
	2.5×10^{-1}	5	16.0	0.38	0.063
	3.0×10^{-1}	5	13.5	2.08	0.074
	3.5×10^{-1}	5	8.57	0.80	0.117
	4.0×10^{-1}	5	6.65	0.98	0.150
	5.0×10^{-1}	5	4.69	1.36	0.213
Lidocaine hydrochloride	1.0×10^{-1}	5	15.2	5.76	0.066
	1.5×10^{-1}	5	9.28	0.84	0.108
	2.0×10^{-1}	5	5.78	1.08	0.173
	2.5×10^{-1}	5	5.41	0.46	0.185
	3.0×10^{-1}	5	4.01	0.41	0.249
	3.5×10^{-1}	5	3.01	0.62	0.332
	4.0×10^{-1}	5	2.58	0.52	0.388
Tetracaine hydrochloride	1.5×10^{-3}	5	120.	28.3	0.008
	2.0×10^{-3}	5	30.3	8.57	0.033
	2.5×10^{-3}	5	20.5	2.58	0.049
	3.0×10^{-3}	5	12.8	1.91	0.078
	3.5×10^{-3}	5	11.1	2.10	0.090
Dibucaine hydrochloride	1.0×10^{-3}	5	45.1	9.04	0.022
	2.0×10^{-3}	5	24.1	4.13	0.041
	2.5×10^{-3}	5	21.8	2.95	0.046
	3.0×10^{-3}	5	17.9	2.04	0.056
	4.0×10^{-3}	5	13.0	3.81	0.077
	5.0×10^{-3}	5	8.65	3.01	0.116

hydrochloride[1] in pH 8.0 phosphate buffer (0.01 *M*) was determined. The solutions were freshly prepared before each experiment. At least five goldfish were used at each local anesthetic drug concentration. The goldfish tank and test solutions were maintained at 22 ± 2°.

Partition coefficients of each local anesthetic agent were determined between chloroform and pH 8.0 phosphate buffer (0.01 *M*). Fifteen milliliters of organic phase and 15 ml of aqueous phase containing the local anesthetics were placed in glass bottles and shaken[3] until equilibrium was established by repetitive sampling. Assay for drug in the aqueous phase after appropriate dilution was done spectrophotometrically[4] at 288 nm for procaine, 262 nm for lidocaine, 310 nm for tetracaine, and 328 nm for dibucaine. All determinations were made at room temperature (22–24°).

RESULTS AND DISCUSSION

Inspection of Table 1 and Fig. 1 reveals that good agreement exists with the four local anesthetic agents tested between the reciprocal of overturn time (1/OT) and drug concentration (4). The data were analyzed by linear regression, and the intercept on the *x*-axis was calculated for each local anesthetic. This value was taken as the apparent minimum effective concentration (MEC) necessary to produce overturn in the goldfish. While it has been shown (5) that the relationship between 1/OT and concentration is not linear at concentrations of drug near the MEC but is hyperbolic, it was felt that this extrapolation would not lead to a significant error in the determination of the MEC. The apparent MEC values for the local anesthetic agents investigated in the present study are listed in Table II.

According to the values listed in Table II, the potency of the local anesthetics in producing overturn in goldfish increases in the following order: procaine < lidocaine < tetracaine < dibucaine. This order of activity is similar to previous results reported for these anesthetics on isolated nerve preparations (6) and in clinical evaluations in human subjects (7).

Agin *et al.* (6), in a report on the action of anesthetic agents on excitable membranes, tabulated the minimum blocking concentration (MBC) of the anesthetic agents necessary to produce conduction blockage on isolated nerve or muscle fibers. These values for

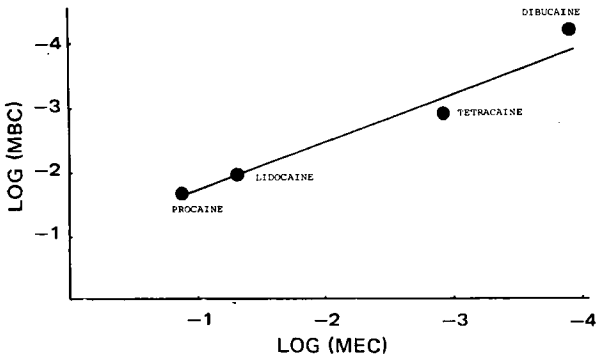

Figure 2—*Plot of the logarithm of the minimum blocking concentration (MBC) of local anesthetics on isolated nerve and muscle fibers* versus *the logarithm of the minimum effective concentration (MEC) of these agents in goldfish. (Data for MBC were obtained from Ref. 6.)*

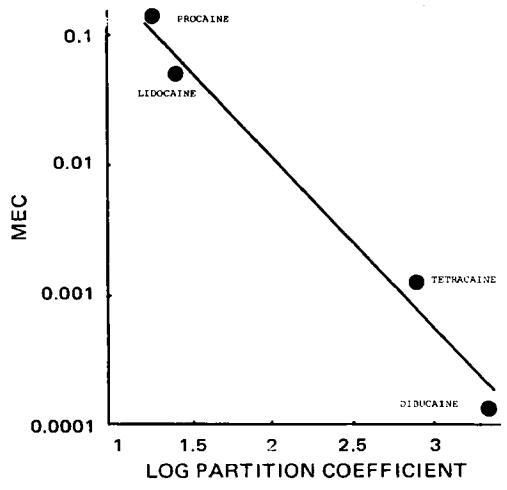

Figure 3—*Plot of the logarithm of the minimum effective concentration (MEC) of local anesthetics in goldfish* versus *the logarithm of the partition coefficient.*

[3] Precision water bath shaker model 25, Precision Scientific Co.
[4] Beckman DB-GT, Beckman Instruments.

Table II—Minimum Effective Concentrations (MEC) in Goldfish, Minimum Blocking Concentrations (MBC) in Isolated Fibers, and Partition Coefficients of Local Anesthetics

Local Anesthetic	MEC, m*M*	Log MBC, m*M*[a]	Partition Coefficient[b]
Procaine hydrochloride	0.136	−1.67	19
Lidocaine hydrochloride	0.049	−1.96	41
Tetracaine hydrochloride	0.0012	−2.9	800
Dibucaine hydrochloride	0.00013	−4.2	2200

[a] Data obtained from Ref. 6. [b] Between chloroform and pH 8 buffer.

procaine, lidocaine, tetracaine, and dibucaine are listed in Table II. A comparison of the results of the present study with those of Agin *et al.* (6) are presented in Fig. 2; the agreement between the two sets of data is good, and the results of present experiments utilizing the goldfish would give a good prediction as to the effect of the local anesthetics on isolated nerve and muscle fibers.

The correlation of biological activity with physical–chemical properties of the drug has been well documented (8). To determine the role of lipid solubility on the effect of the local anesthetic agent on overturn time in goldfish, partition coefficients between chloroform and pH 8 buffer were measured (Table II). The order of increasing lipid solubility of the agents between chloroform and pH 8 buffer is as follows: procaine < lidocaine < tetracaine < dibucaine. The relationship between partition coefficient and the apparent MEC is illustrated in Fig. 3. The fit is rather good and demonstrates that the activity of the local anesthetics in producing overturn in goldfish is due, in part, to the lipophilicity of the drug molecule.

Experimental results also appear to indicate that the nonionized drug molecule is responsible for the observed effect of the anesthetics on overturn time. In an experiment with lidocaine where the pH of the buffer solution was varied, the mean overturn time in goldfish for a 0.4 m*M* solution was 2.6 min at pH 8 and 16.8 min at pH 7; there was no response at pH 6. Lidocaine, with a pKa of approximately 7.9 (9), is 55.7% unionized at pH 8, 11.2% unionized at pH 7, and 1.2% unionized at pH 6. Thus, with decreasing pH and the subsequent decrease in in the concentration of unionized drug and the ability of the drug to penetrate biological membranes, there is a reduction in the pharmacological activity of lidocaine in the goldfish. A similar effect of pH on the overturn of goldfish in the presence of the other anesthetics investigated was noted.

The results of the present study illustrated the potential usefulness of goldfish as a model for the prediction of drug activity. Investigations are currently underway concerning the structure–activity relationships involved in producing overturn and death in goldfish for a series of lidocaine derivatives.

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Characterization of Poison Oak Urushiol

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Abstract □ Procedures are described that were used in the isolation and characterization of urushiol components reported to be the allergenic constituents of poison oak, *Toxicodendron diversilobum*. Characterization of these components by spectral techniques indicated they are unsaturated congeners of 3-heptadecylcatechol, possessing one, two, or three double bonds in an unbranched C_{17} side chain. These components are shown to differ from those isolated from poison ivy, *Toxicodendron radicans*, by a $—CH_2CH_2—$ unit in the unbranched alkyl side chain.

Keyphrases □ Poison oak—isolation and characterization of urushiol components □ *Toxicodendron diversilobum*—isolation and characterization of urushiol components □ Urushiol components of poison oak (*Toxicodendron diversilobum*)—isolation and characterization

Plants of the genus *Toxicodendron* (Anacardiaceae) have long been known for their ability to produce contact dermatitis in susceptible individuals. The best known species of this genus in the United States are *Toxicodendron radicans* (poison ivy), *Toxicodendron diversilobum* (western poison oak), and *Toxicodendron vernix* (poison sumac). The systematics of this genus have been well characterized (1).

The composition of the allergenic urushiol components (2–4) of poison oak (*T. diversilobum*) was studied. A prior study of the urushiol content of poison