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FURTHER OBSERVATIONS ON THE PHARMACOLOGY OF ESTERS OF M-AMINOBENZOATE IN DOGFISH: LOCAL vs GENERAL ANESTHESIA

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In previous studies we found that ethyl-m-aminobenzoate (MS 222) did not cause "anesthesia" in the dogfish by the parenteral route, although sedation by gill intake is well known in this and other fish and "lower" vertebrates. Correlated with this appeared to be its exclusion from brain following injection; surprising in view of its lipid solubility but explicable on the grounds of very high clearance from gill (Bull. MDIBL 7:51, 1967).

The isobutyl homologue of MS 222 (called IBA) was found to be about eight times as active in repressing the tail reflex of goldfish, when the drugs were added to the ambient water. Furthermore, IBA caused sedation in the dogfish following intravenous injection in the range 20 - 50 mg/kg (Bull MDIBL 8:42, 1968). The present experiments were designed to find the pharmacologic basis for the difference between the two drugs, and explore the possibility of using IBA as a general "anesthetic" in fish. Unexpectedly, the results suggest that neither drug acts centrally, but are local anesthetics; facts in closer conformity to their chemical structure.

IBA caused the fish to lie quietly in the live car, with normal respiration, following intravascular dose of 35 mg/kg or intramuscular dose of 60 mg/kg. The vascular dose usually caused brief excitement, followed by quieting which lasted several hours. The intramuscular dose provided a surprisingly rapid onset; 1-5 minutes, followed by quieting for several hours. Higher doses were toxic by either route, and lethal over 50 mg/kg i.v. or 100 mg/kg i.m. An unexpected manifestation of toxicity (not observed with MS 222) following IBA by injection at sedative doses was slight to moderate hemolysis of red cells. This, as well as the rather narrow margin of safety, turned off further development of this drug.

Cerebrospinal fluid was analyzed for IBA after injection (30 mg/kg i.v.) over a period of 3 hours while the fish was in a divided box with the head arranged for continuous sampling or while the fish was swimming freely in the live car. In no case (of 11) was drug ever found (less than 2 μg/ml) even when plasma concentration was as high as 400 μg/ml. This was similar to data cited above for MS 222.

It seemed evident that the "sedation" was in fact a nerve-muscle block, rather than central anesthesia. Analysis was made of our earlier data in which the distribution of MS 222 in blood and brain was studied following its uptake from the gill and rapid sedation (Stenger and Maren, in press, and Bull. MDIBL 7:51, 1967). A distinguishing feature of these data (as compared with those from intravascular injection which did not cause sedation) is the immediate and sustained equilibration of drug in arterial plasma with that in ambient water. Such plasma levels were higher than that possible following injection in the free swimming fish. Cessation of anesthesia when the fish was returned to drug-free water could best be correlated with rapid disappearance of drug from blood.

IBA also caused rapid (2-5 minutes) sedation when fish breathed water containing 10^{-4} drug. It appeared somewhat more active than MS 222, like the data from goldfish cited above, and in accord with its heavier alkyl chain. This higher activity explains why IBA causes "sedation" by the parenteral route (in contrast to MS 222): it can elicit motor paralysis at arterial plasma con-

centrations of about 20 μg/ml, while 50-100 μg/ml is required for MS 222.

Dr. T. F. Muther of this department had independent evidence that neither MS 222 nor IBA have central action, but that "anesthesia" is due to blockade of peripheral neurotransmission or neuromuscular junction or both. He stimulated the posterior part of the spinal column of goldfish electrically. There was a correlation between the decrease in tail reflex contractions and depth of anesthesia as measured by rate of opercular movements.

It is of passing interest that MS 222 has been regarded as general central anesthetic for cold-blooded animals for 50 years. There appears to be a serious information and communication gap between warm-blooded investigators and the poikilotherms.

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TRANSFER RATES OF CO_2 AND CL^- FROM PLASMA TO CEREBROSPINAL FLUID (CSF) IN Squalus acanthias: EFFECT OF CARBONIC ANHYDRASE INHIBITION

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We wished to find the rate at which the major anions are transferred from plasma to CSF. $NaH^{14}CO_3$ and $Na^{36}Cl$ were injected into the caudal artery, and the accumulation of label in the cerebellar ventricle of the dogfish was measured. Cold concentrations of these anions were also monitored, along with appropriate measurements of anions in the plasma. Carbonic anhydrase was inhibited by injecting acetazolamide (30 mg/kg) intravascularly 15-30 minutes before injection of the label. Enzyme in choroid plexus, as well as in gill and red cells, will be completely inhibited by this procedure (Physiol. Rev. 47:595, 1967).

CO_2. Two different protocols were used. (1) Fish in the box, with continuous perfusion of the gills (Comp. Biochem. Physiol. 26:853, 1968) and small opening in the skull, through which CSF could be sampled at intervals. (2) Fish in 25 gallon tank of fresh sea water, freely swimming. CSF was sampled 6 minutes after injection of label. The usual dose of carbon label was 40 μcuries.

1. Table 1 gives data from the box experiment. The concentration of labeled CO_2 (all forms) in the three minute control (untreated) plasma sample is set as 100, and all other counts are relative to this. Up to 12 minutes CO_2 disappearance rate from plasma is like that in the free swimming fish (see below) and in accord with the rate of metabolism and volumes of fluid recorded by Robin and Murdaugh (Sharks, Skates and Rays, ed. by Gilbert et al, Baltimore: Johns Hopkins Press, 1967). In the acetazolamide-treated animals, this rate is unchanged, but there is an initial retention of the label, leading to higher total CO_2 counts; also there is a higher proportion of gaseous CO_2. This leads to a much higher concentration of labeled gaseous CO_2 than controls (last column). This is the expected effect of carbonic anhydrase inhibition in this species (Comp. Biochem. Physiol. 5:201, 1962).

The uptake of total CO_2 into the CSF was roughly linear for the first 12 minutes of the experiment, when it reached the level in the plasma. To analyze the role of carbonic anhydrase in CO_2 transport the rates of transfer from plasma to CSF in the control and acetazolamide treated animals were compared. In each case, these rates were related to the concentration of gaseous