

Morphine Metabolism and Morphine Tolerance in Goldfish

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Tolerance to morphine was induced in goldfish shown to be unable to metabolize morphine. Ability to metabolize morphine is, therefore, not a prerequisite to development of tolerance. (Key words: Morphine; Tolerance.)

REPEATED ADMINISTRATION of a narcotic analgesic is associated with development of tolerance. The tolerant animal shows progressively less response to small doses of narcotic and can tolerate large doses without evidence of toxicity. It is not clear how or why tolerance develops. Some suggest that the sensitivity of the cell receptors upon which narcotics act is altered in the tolerant animal,¹ and that the development of tolerance is associated with alterations in the rate or pathway of drug metabolism.¹ The role of altered drug metabolism in development of tolerance has been questioned but not disproven. It occurred to us that if tolerance could be induced in an organism incapable of metabolizing narcotics, then drug metabolism would not be a prerequisite to development of tolerance.

We selected the common goldfish (*Carnasius auratus*) for study because although other fish possess microsomal enzymes capable of biotransforming lipid-soluble foreign compounds,^{2,3} goldfish can absorb lipid-soluble substances such as narcotics, but cannot metabolize them.^{4,5} Our study consisted of three parts: 1) proof that a narcotic (morphine) is taken up by the goldfish; 2) determination whether morphine, once absorbed, is

metabolized; 3) determination whether goldfish become tolerant to morphine following repeated exposure.

Experiment 1

A colony of fish, Comet strain, each measuring 6-7 cm in length and weighing 24-35 gm, was established and acclimatized for a period of weeks, particular attention being paid to diet, quality and temperature of water, and amount of exposure to daylight. Five pairs of fish were studied, each pair consisting of an experimental and a control fish. The study consisted of placing an experimental and a control fish in separate plastic containers with 200 ml of constant-temperature, aerated aquarium water, with a third container without fish serving as an additional control. Morphine sulfate (2,000 µg, without preservative) was added to the container holding the experimental fish and to the control container without a fish. Morphine was absorbed via the gills as evidenced by behavioral responses induced (v.i.). To measure uptake of morphine the water was removed from the containers after two hours and analyzed colorimetrically for phenolic compounds by the method of Fujimoto.⁶ The fish were preserved for further study by the immediate addition of 100 ml of fresh aquarium water. The aquarium water contained no phenolic compounds. Therefore, the total amount of phenol in the water of the experimental fish minus the trace amounts of phenol excreted into the water by the control fish represented morphine. Morphine uptake was calculated by subtracting from the amount of morphine added to the water the amount present two hours later. The absence of change in morphine concentration in the container without fish demonstrated that the plastic walls of the containers did not take up morphine.

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TABLE 1. Morphine Uptake in Five Goldfish

| Fish | Total Uptake (μ R) | Uptake/Weight (μ g/gm) | Total Recovered (μ S) | Per cent Recovery |
|------|-------------------------|-----------------------------|----------------------------|-------------------|
| 1 | 280 | 9.3 | 280 | 100 |
| 2 | 260 | 7.5 | 260 | 100 |
| 3 | 240 | 9.6 | 230 | 96 |
| 4 | 320 | 9.4 | 333 | 103 |
| 5 | 420 | 13.0 | 420 | 100 |

Uptake of morphine averaged 9.6 μ g/gm body weight (table 1). By altering the duration of exposure to morphine, we found that absorption was completed in approximately 15 minutes, no further uptake occurring during the next three hours.

Experiment 2

To measure the amount of morphine metabolized we reversed the uptake process by repeatedly exposing the fish to fresh water and measuring the total amount of unaltered morphine recovered. This was done by taking the fish exposed to morphine for two hours and exposing it for 30 minutes to each of four successive 100-ml washings of fresh aquarium water. These 100-ml samples were then placed in a volumetric flask and analyzed for phenols by the method of Fujimoto.⁶

Conjugation with glucuronic acid at the phenolic site of the morphine molecule is the predominant metabolic pathway by which morphine is inactivated in most species. By measuring the concentration of phenols in water we could calculate the amount of morphine which had not undergone conversion to 3-morphine glucuronide. This was done by subtracting the total amount of morphine recovered in phenolic form in the four successive 100-ml washings from the total amount of morphine taken up by the fish during its two-hour exposure to morphine. Recovery rates determined this way ranged from 96 to 103 per cent (table 1). We concluded that goldfish did not degrade morphine by conjugation. However, morphine can also be metabolized by processes affecting the molecule at other sites. Metabolism by N-demethylation to normorphine was sought, using both thin-layer chromatography⁷ and ultraviolet spectrophotometry,⁸ after preliminary tests using

stock solutions of normorphine showed that quantities of normorphine as low as 0.5 μ g/ml could be detected. Analysis of washings by these techniques revealed no normorphine. We conclude that goldfish do not metabolize morphine by conjugation, by N-demethylation, or in any other way.

Experiment 3

To determine whether tolerance to morphine could be induced, experimental and control fish of the same size were placed in separate plastic containers (23 \times 7 \times 7 cm) with one liter of aerated, constant-temperature water. An electric prod⁹ provided an accurate and reproducible painful stimulus of graded intensity immediately caudad to the dorsal fin. The end-point was appearance of an agitated swimming response (ASR), consisting of a sudden, pronounced increase in frequency and amplitude of fin and opercular movements. By gradually increasing voltage, thresholds for ASR were determined in both experimental and control fish. (Threshold stimuli for all fish averaged 4.75v.) Morphine was then added to the water of the experimental fish in increments of 140 μ g until ASR at threshold stimulus was repressed, *i.e.*, until analgesia to the painful stimulus was achieved. The potential was then increased until ASR was re-established, following which further morphine was added until ASR was again eliminated. By continuing the stepwise increases in voltage and morphine in this manner dose-response curves were obtained (fig. 1). The upper parts of these curves were "leveled off." At the higher doses of morphine fish became hyperactive and began to swim in a bizarre, manic manner in the absence of electrical stimulation. Control fish remained quiescent in their containers throughout the period of testing unless stimulated with the electrical prod, and showed no change in threshold stimulus upon repeated testing. Dose-response data were obtained over a period of six to eight hours. The fish were then allowed to rest, after which the same experimental fish and their paired controls were subjected to the same procedure every three days for three to four times. Thus, dose-response curves were obtained in the presence and absence of re-

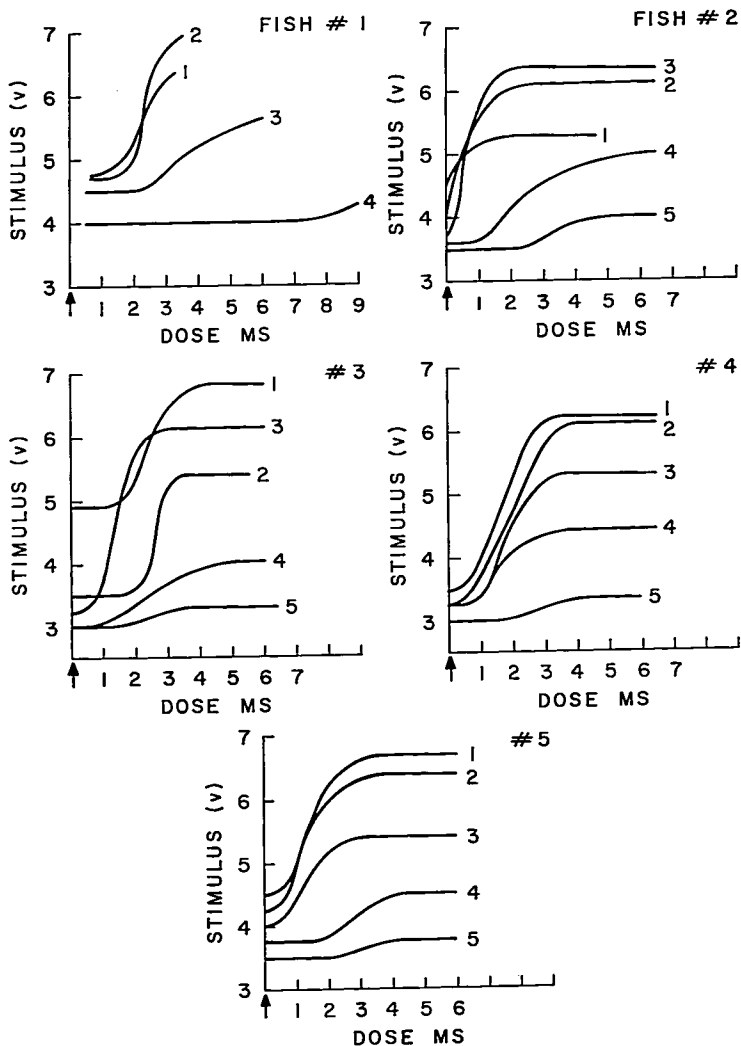


FIG. 1. Electrical stimulus (in volts) required to produce agitated swimming response with increasing increments (140 μ g each) of morphine when goldfish are exposed to morphine repeatedly (as indicated by numbered curves). Arrow indicates threshold stimulus in the absence of morphine.

peated exposure to morphine. The threshold stimulus to produce ASR in unmedicated fish remained unchanged over the course of repeated experiments. However, with successive exposure to morphine there was a shift of the dose-response curves downward and to the right. This shift indicated development of tolerance to the analgesic effect of morphine: the same dose of morphine repeatedly administered no longer had the analgesic effect as when administered the first time. In other words, increasing doses of morphine were required to provide analgesia to the same electrical stimulus. Two other phenomena also indicated development of tolerance to other actions of morphine. Hyperactive, agitated swimming motions normally observed with exposure to high doses of morphine became less and less pronounced when morphine was administered repeatedly until finally this type of behavioral response could no longer be produced with any dose of morphine. Second, morphine regularly resulted in the passage of feces in nontolerant fish. This effect also disappeared upon repeated exposure to morphine.

All five fish made tolerant to morphine were tested by the methods described above to determine whether while tolerant, they could metabolize morphine; none could.

Discussion

Although the association of tolerance and altered metabolism of narcotics has been questioned,^{10,11} there are many reports indicating that animals tolerant to narcotics are unable to metabolize at normal rates or by normal pathways the drugs to which they are tolerant. In nontolerant dogs, for example, 80 to 92 per cent of the morphine administered can be recovered in the urine, while in tolerant dogs only 33 to 66 per cent can be recovered.¹² Liver slices from tolerant rats conjugate morphine more rapidly than liver slices from nontolerant animals.¹³ On the other hand, chronic administration of morphine to rats decreases the activity of hepatic microsomal enzymes responsible for N-demethylation of morphine.^{14,15} Recently, it has been shown¹⁶ that rats tolerant to dihydromorphine show marked impairment of the ability to demethyl-

ate labeled N-¹⁴C-methyl-dihydromorphine. In control animals 3.7 per cent of the injected radioactivity can be recovered as ¹⁴CO₂, while in animals which have received daily injection of the drug for three weeks only 0.5 per cent of injected radioactivity can be recovered as ¹⁴CO₂. Furthermore, animals treated for seven weeks with dihydromorphine return to normal, nontolerant levels of ¹⁴CO₂ excretion within nine days of cessation of drug administration.

The above data could mean that development of tolerance depends upon and is the result of inability of the tolerant animal to metabolize narcotics normally. The data could also mean that abnormal narcotic metabolism occurs coincident with development of tolerance, and that no true causal relationship exists between the two phenomena. That the goldfish in the present investigation were unable to metabolize morphine and yet were able to develop tolerance to morphine indicates that, in goldfish at least, tolerance is not the result of altered metabolism. The present report supports the conclusion that no causal relationship exists between development of tolerance and altered metabolism, since synthetic compounds molecularly similar to morphine, some with and some without analgesic effects, do not have stereospecificity in terms of their effects on drug-metabolizing enzymes.^{17,18}

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Surgery

MYOCARDIAL INFARCTION One hundred and forty-one randomly-selected surgical patients, 35 years old or older, were studied preoperatively, followed through their operative procedures, and reassessed during the first postoperative week for evidence of myocardial ischemia associated with surgical operations under general anesthesia with nitrous oxide with or without halothane. Of these patients, 38 per cent had preoperative clinical evidence of heart disease, hypertension or diabetes; 45 per cent had abnormal preoperative electrocardiographic patterns. Three patients, all in the sixth decade of life, experienced silent myocardial infarctions during or within 36 hours of operation; all were in the abnormal group preoperatively, one having nonspecific EKG changes, one having an arrhythmia, and one having left ventricular hypertrophy. In two, the diagnosis of infarction was indicated by changes in the EKG and in one, by enzyme studies. Significant postoperative EKG changes were found in 17 per cent of the normal and 18 per cent of the abnormal preoperative groups. A relationship existed between the rise in serum lactic dehydrogenase concentration and the field of operation, but the diagnosis of infarction was not in doubt provided serum lactic dehydrogenase isoenzyme patterns and increase in serum aspartate aminotransferase levels were consistent with the diagnosis. (Hunter, P. R., and others: *Myocardial Infarction Following Surgical Operations*, *Brit. Med. J.* 4: 725 (Dec.) 1968.)