BENZOCAINE: AN EXCELLENT AMPHIBIAN ANESTHETIC

For some time, the anesthetic of choice for amphibians has been MS-222, the methane sulfonate salt of ethyl m-aminobenzoate. (See, for instance, Hamburger, 1960; Rugh, 1962.) MS-222 has also been used widely in the anesthesia of fish (Bove, 1962; Wayson _et al._, 1976). Recommended concentrations of this anesthetic for amphibian embryos are in the range of 0.02% to 0.03% (1:5000 to 1:3000), but in our hands, to have reliable anesthesia for measuring adult newt stump currents with the ultrasensitive vibrating probe (which, at that time, was easily smashed with just one twitch of a stump), a concentration of 0.35% was necessary (Borgens _et al._, 1977). We found, moreover, that repeated MS-222 anesthesia of newts with this concentration (which was necessary in order to read stump currents every other day or so) was debilitating to the animals. We also found that the action of MS-222 was often irregular: Sometimes two or three times the normal time of 10 minutes was necessary for adequate anesthesia.

In view of these difficulties with MS-222, I was delighted to discover some years ago that benzocaine (listed just below MS-222 in the Sigma catalogue) is actually ethyl p-aminobenzoate, a closely-related isomer of MS-222. It also was immediately clear that it is far less expensive than this troublesome anesthetic: At the present time, its cost per 100 grams from Sigma is 7 times less than the cost of MS-222. It seemed logical to try some, and indeed it was. We have used benzocaine exclusively as our amphibian anesthetic for the past 7 years.

We quickly found that 0.02% benzocaine is the equivalent of 0.35% MS-222 in anesthetizing _Notophthalmus viridescens_, further increasing the economy of using it instead of MS-222. In due time, it became apparent that other species of amphibians, including the IU axolotls that have contributed much to our research efforts, can also be successfuly anesthetized with this compound. The species with which we so far have had experience (see Borgens _et al._, 1984; Vanable _et al._, 1983) include:

- _Ambystoma mexicanum_
- _Aneides lugubris_
- _Batrachoceps attenuatus_
- _Desmognathus quadramaculatus_
- _Ensatina escholtzi xanthoptica_
- _Necturus maculosus_
- _Notophthalmus viridescens_
- _Pleurodeles watl_
- _Rana pipiens_ (adults)
- _Taricha granulosa_
- _Taricha torosa_
- _Xenopus laevis_ (larvae & juveniles

Benzocaine dissolves slowly in water. Therefore, it is expedient to first dissolve it in absolute ethanol, and then add whatever solution is appropriate for the animals being studied, to dilute the benzocaine to the proper concentration. (Our artificial pond water is 1.5 mM NaCl, 0.06 mM KCl, and 1.0 mM $CaCl_2$.) The final concentration of ethanol is 1%. For example, to make a liter of 0.02% benzocaine solution, we dissolve 0.2 g of benzocaine in 10 ml absolute ethanol (benzene-free), add 10 ml of a 100X stock solution of our artificial pond water, and then add de-ionized water to make the volume up to 1 liter. Benzocaine solutions are stable at room temperature for at least two weeks.

The concentration of benzocaine that is needed for effective anesthesia varies somewhat with the size of the animal to be anesthetized. Our rule of thumb has been to adjust the concentration of benzocaine so that anesthesia is complete after about 10 minutes of immersion. (The end point is failure to execute the righting reflex after turning the animal on its back.) It is rare to have large deviations in the length of time needed for anesthesia. For Notophthalmus and other species of amphibia of similar size, 0.02% benzocaine is suitable. For larger axolotls and adult Rana pipiens, 0.03% benzocaine is advisable. For larvae of Xenopus laevis (and, presumably, for other small larvae), much lower concentrations must be used: 0.001% to 0.005%, depending on the stage of the larva.

The time of recovery from benzocaine anesthesia depends on whether the animal is returned to anesthesia-free water, or kept (moist) out of water. When returned to water, most animals recover in 45 to 60 minutes. Xenopus larvae recover quite rapidly; it is often necessary to keep them in 0.001% benzocaine while making current measurements, to keep them adequately anesthetized. Axolotls and Taricha kept wrapped in moist gauze during the course of a complex electrode implantation remain anesthetized for two hours. Recovery from proper levels of anesthesia (10 minutes' immersion in a concentration of benzocaine that eliminates the righting reflex by this time) is complete; repeated anesthesia every other day for at least two weeks has no discernible debilitating effect. We are now beginning to gain experience with keeping Notophtahlmus viridescens ansesthetized continuously for a whole day, in moist condition, with a small patch of benzocaine powder (ca. 1 mg) on the trunk skin. We have, however, too little experience with this to make a clear recommendation at this time.

The action of benzocaine is reasonably well understood: It quickly and reversibly blocks sodium channels in the axon mem brane (Strichartz, 1976; Hille, 1977; Neumcke et al., 1981). It is clear that axon membrane sodium channels are distinctly different from the sodium channels in the outer membranes of amphibian epidermal cells: Benzocaine has no effect on the stump currents that are dependent on sodium's diffusion through these epidermal channels.

In sum, benzocaine is a reliable, non-debilitating, stable and inexpensive anesthetic for a wide range of amphibian species, both urodele and anuran. In our hands, this compound has proved to be distinctly preferable to the widely known amphibian anesthetic, MS-222. In view of MS-222's wide use in fish anesthesia, both in research and in industry, it should be worthwhile to see whether fish do as well with benzocaine as do amphibians.

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