Targeting Talk: *Anti-DBH-SAP Administration*

by Dr. Sue Ritter, Guest Contributor and anti-DBH-SAP expert

Q: We injected anti-DBH-SAP into the hypothalamus of Sprague-Dawley rats and sacrificed them 2 weeks later. We did not see any reduction in the DBH fiber staining.

When the drug arrived, we aliquoted it in 1-µl snap-cap tubes on ice, and stored them at -80°C. For injections, a 1-µl aliquot was diluted to a little over 10 µl so that we had a final concentration of 1 µg/10 µl.

We administered two injections of 100 nl on each side (with 10 ng of anti-DBH-SAP) using a 0.5-µl Hamilton syringe attached to a stereotax. The needle was a 33-gauge with a blunt tip. I tried previously to use glass micropipette tips attached to a Hamilton syringe with the line filled with mineral oil, but found that the actual volume displacement was too unreliable.

A: We have not had any problems related to the stability of anti-DBH-SAP. In our work, failure to lesion is nearly always associated with a misplaced injection. From the information conveyed, I would suggest the following:

(1) It is possible that no drug was actually delivered to the brain. Two things could be done to ensure drug delivery. The first would be to add a tracer to the saporin solution that could be identified histologically. The second would be to visually monitor drug delivery using a calibrated tip. Air bubbles, pressure leaks and compression of the liquid can interfere with accurate delivery.

(2) It is possible that the anti-DBH-SAP was not delivered to the correct site, so that the expected uptake into the targeted terminals did not occur. Again, marking the site so it is clear where the injection was would help evaluate your accuracy. Establishing a reliable set of stereotaxic coordinates that work in your lab, in your rats and with your equipment and then using a dye to estimate the diffusion radius of your selected injection volume are always good ways to start. However, that being said, it should not be difficult to locate the injection site with such a large injector (33 g) - so #1 seems more likely to be the problem in the case you describe. Also, I would add that the larger the injector, the more nonspecific damage there will be. Glass capillary micropipettes are by far preferable to stainless steel cannulas in providing more reliable delivery of small volumes and in producing less nonspecific damage. Chronically implanted cannulas should be avoided, in my opinion, because gliosis at the cannula tip is apt to occur and this may alter the diffusion pattern of the injected substance, as well as interfering with lesion analysis.

(3) Try a different anesthetic. We have not tested a lot of anesthetics, but we have had problems getting a good lesion that we think are attributable to use of a ketamine/xylazine/acepromazine anesthetic cocktail. So we routinely avoid that one.

(4) I assume you are looking at fibers in the area of the injection. If not, it would be important to make sure the fibers being evaluated are associated with the same neurons innervating the terminal field at the injection site. Secondly, the 2-week wait mentioned between toxin injection and histology is critical for evaluating the lesion to assure that immunoreactive products are no longer present. Making sure that tissue processing controls are stringently adhered to so that controls and lesioned animals are run together in the same batch is also important.

(5) You might try injecting only one side and comparing terminal staining with the non-injected side in the same animal. This would not be a good idea, however, if the injection site is too close to the midline, so that both sides might be damaged from a unilateral injection.

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