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IN VIVO MICRODIALYSIS

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Patrick Mason and D. Bhaskaran are Instructors of Medicine at the U of C Health Science Center. Working with Curt R. Freed, M.D., they use in vivo microdialysis and in vivo electochemistry to investigate the manner in which changes in plasma osmolality and blood pressure and volume alter the cental concentrations ofcatecholamines, indo-leamines, and ascorbic and uric acids.

In vivo microdialysis is an exciting new technique with which to explore the relationship between brain and behavior. This technique permits the researcher to monitor continuously the extracellular fluid concentrations of catechola-mincs, indoleamines, amino acids, and peptides in the brain. The technique of in vivo microdialysis is similar to the push-pull cannula technique (Gaddum, 1961) except that a semipcrmeable membrane is attached to the tip of the cannula (see Figure 1). This membrane protects the surrounding tissue from being damaged by the inflowing per-fusion fluid, as well as permitting only small molecules to be extracted from the tissue. In vivo microdialysis is useful not only for extracting chemicals from brain tissue but also for the slow and continuous infusion of drugs into the brain tissue.

The earliest use of this type of dialysis was by Delgado et al. (1972) and Ungerstedt and Pycock (1974). Ungerstedt and colleagues developed a dialysis probe which is currently marketed through Carnegie-Medicine and Bioanalytical Systems (BAS) and is probably the most popular dialysis probe that is commercially available. This dialysis probe is usually implanted vertically in the brain and consists of two concentric tubes. The smaller inner tube is for the inflow of the perfusion fluid and the larger outer tube is for the outflow of the perfusate. The dialysis membrane is available in various lengths ranging from 2 to 5 mm. Some researchers (Tossman and Ungerstedt, 1981; Hernan-dez et al., 1983; Sharp et al., 1986) have used a dialysis membrane which forms a half loop between two adjacent tubes. Inside the dialysis membrane is a fine thread to prevent the dialysis membrane from collapsing. Other researchers (Ungerstedt et al., 1982; Imperato and Di Chiara, 1984; Zetterstrom and Ungerstedt, 1984; L'Heureux et al., 1986; Console et al., 1987) have used a dialysis membrane that was placed horizontally in the brain. Portions of the dialysis membrane's outer surface were coated with glue in order to make the membrane selective as to which region it dialyzed.

The most important and delicate portion of the dialysis probe is the semipermeable membrane which is usually made of cellulose. Dialysis membranes are available from several companies (Amicon Corporation; Cole Farmer Instruments Company; DOW Company; Spectrum Medical Industries). Depending upon the manufacturer's specifications, the maximum size of molecule that passes through the membrane varies between 1,000 and 12,500 daltons and the diameter of the membrane varies between 200 and 850 fim. The diameter and length of the dialysis membrane used in an experiment should depend upon the width and length of the brain nucleus to be dialyzed. The length of the membrane is usually between 2 and 5 mm, but we cur-

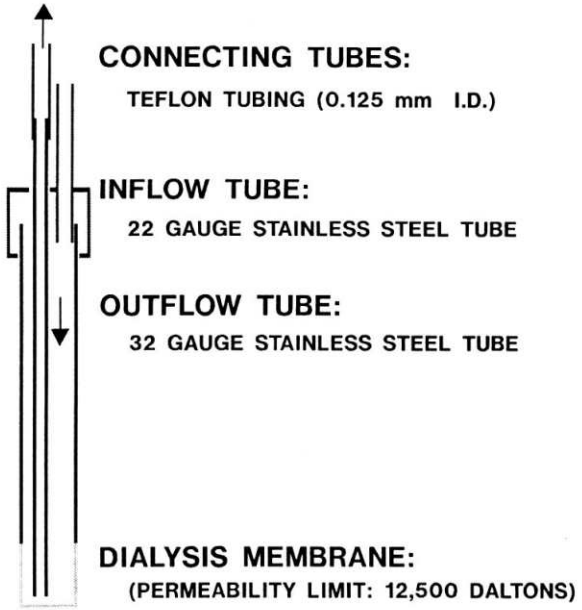

Figure 1: Shown is the design of our *in vivo* microdialysis probe. The outer 22 gauge tube is for the inflow of the perfusion fluid. The inner 32 gauge tube is for the outflow of the perfusate.

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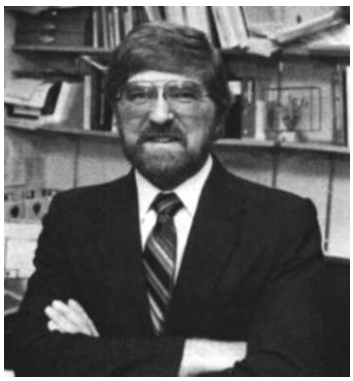

Editor's Column

It doesn't seem like it could be almost time for the next Neurosci-ence Convention. The summer has gone by so fast and now fall is in the air. I do hope that many of you will stop by the Kopf booth in Toronto to visit with the folks from the company and with me while you are at the convention. It is always great to see you and to have your comments about the Carrier and what you would like to see in it I welcome your ideas for articles and topics to appear in the newsletter. Also, please consider writing an article for publication in the Carrier and stop by the Kopf booth at the convention to talk with me about your ideas for articles or your article. Remember, there is an honorarium for each article published and the newsletter has a circulation of over 10,000. If you cannot be at the Neuroscience Convention, just call or drop me a line at the address below.

The article in this issue is a very interesting one about a relatively new technique which has many possibilities. I urge you to read it and think about how the technique could benefit you. It is amazing how rapidly new techniques are coming along in the area of neuroscience. It makes one wonder if one can keep up.

In the past few months, there seems to have been more and more publicity about animal rights activists and more pressure on legislators and federal officials to restrict animal research. This is a very critical issue for those of us engaged in animal research, since the avowed purpose of many of these people is to stop all animal use in research. We must become more active ourselves in voicing our side of the research story and the benefits of our work. We are just beginning to understand much about brain function and learning. What would happen if we could no longer study these processes in animals? What would happen to the momentum in underslanding mental disease? On the other side of the coin, we must be very careful of our treatment of animal subjects, remembering that these are organisms which can feel and experience. To continue to use animal subjects, we must assure responsible use. Be active in the debate; it is important.

See you in Toronto-Stop by to see me at the Kopf Boodi or leave a message there.

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trently use membranes as short as 0.5 mm. Decreasing the total surface area of the membrane attenuates the absolute amount of neurochemical extracted. However, a small dialysis membrane is essential when working with the smaller brain nuclei so as to avoid contamination from surrounding nuclei.

New membranes are usually filled with glycerol (3-5%). Glycerol prevents the membrane from becoming dry and brittle when exposed to the air. During the initial use, the glycerol is gradually replaced by the perfusion fluid. At the end of the experiments, the dialysis probe should be perfused with and stored in double-distilled water. Since some membrane material does not provide as good recovery on subsequent uses as it does in the initial use, we replace these dialysis membranes after each experiment. To rebuild"" our dialysis probes we simply ream the inside of the larger outer tube with a 29 gauge tube and then slide a new membrane over the smaller inner tube. The tip of die membrane is sealed with epoxy (Devcon Corp., Danvers, Mass.)-Once the epoxy has dried we gently push the tip of the membrane towards the tip of the smaller inner tube and then epoxy the membrane to the tip of the larger outer tube. After through drying, the probes are perfused with and stored in double-distilled water.

Another important component of the dialysis probe is the inflow-outflow design. We prefer to perfuse our probes so that the smaller inner tube is for the outflow. The same flow direction has been used by Robinson and Whishaw (1988). However, this direction of flow may not be feasible with other dialysis probes due to differences in probe design and durability. We perfuse in this direction to decrease the dead volume which exists between the dialysis membrane and the end of the outflow tube. Having the smaller inner tube for the outflow becomes even more important when dialyzing large animals since the length of the outflow tube may become relatively long. Decreasing the length and internal diameter of the outflow tube decreases-the time during which degradation of the extracted neuro-chemicals may occur. When plotting the extracellular fluid neurochemical concentrations against time it is important to subtract the time required for the ncurochemicals to move from the dialysis membrane to the end of the outflow tube.

The rate of perfusion is also very important because it influences not only the relative rate of recovery but also the longevity of the dialysis probe. Increasing the perfusion rate decreases the relative rate of recovery (Johnson and Justice, 1983; Hernandez et al., 1986; Ungerstedt and Hall-strom, 1987). A commonly used perfusion rate is 2.0 |il/ min but the perfusion rate may vary between 0.1 and 10.0 (4,1/min. At the initiation of an experiment, we use a high rate of perfusion to dislodge air bubbles from the dialysis probe. However, a very high perfusion rate (> 10 (il/min) may dislodge the epoxy from the tip of the dialysis membrane or even dislodge the dialysis membrane from the tip

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of the larger outer tube. Since the perfusion rate influences the relative rate of recovery, the infusion rate should not be altered once determination of the baseline concentrations has begun. When perfusing using a glass syringe it is important that it is a gas-tight syringe. Otherwise, the pressure created to push the perfusion fluid through the dialysis probe may push the perfusion fluid out through the back of the syringe. The composition of the perfusion fluid should be compatible with the substance being dialyzed. Physiological Ringer's solution or artificial CSF is commonly used. Changes in the composition of the perfusion fluid may produce variation in the amount of neurochemical released from the tissue (Imperator and DiChiara, 1984; Con-solo et al., 1987).

The method used to determine the concentrations of neu-rochemicals in the perfusate may dictate the manner in which the perfusate is collected. If high pressure liquid chromatography (HPLC) is the method of analysis, the perfusate can be injected directly onto the column using a computer-controlled injector (Wages et al., 1986; Sabol and Freed, in press) or the perfusate can be collected in micro-centrifuge vials and then manually injected onto the column. The former method permits very small volumes of perfusate (1-5 p.1) to be injected onto the column since there is no loss in transfer. If the perfusate is collected into microcentrifuge vials, which would be likely if the perfusate was to be analyzed using radioimmunoassay, the vials should be placed on ice throughout the collection period to prevent degradation of the sample. When assaying for cate-cholamines it has been recommended that a small amount of 0.1 N perchloric acid be placed in the vial to prevent oxidation (Keller et al., 1976; Imperato and DiChiara, 1984; L'Heureux et al., 1986). Due to the extremely low extracellular fluid concentrations of some ncurochemicals, we inject the perfusate onto microbore HPLC columns (100 x 1.0 mm, 3 (im). We increase the signal to noise ratio by using either a Waters pump (Model 510) with a pulse dam-pener or an ISCO microflow syringe pump (Model uLC-500) which is pulseless.

The dialysis probe should be perfused for at least 90 min after implantation and prior to the initiation of baseline collections. The insertion of the dialysis probe or any movement of the probe once it has been inserted can cause large concentrations of some neurochemicals (e.g. dopa-mine) to be released from the surrounding tissue (Imperto and DiChiara, 1984). Several hours may be required before the neurochemical concentrations return to basal levels. With the anesthetized animal this is not a major inconvenience as long as the level of anesthesia and the animal's body temperature is maintained. With the chronic, freely-moving animal any delay will probably increase the frustration level of the researcher.

It seems that the chronic, freely-moving animal always wants to turn in circles during the perfusion period. With the on-line HPLC system, a dual-channel fluid swivel is essential. To prevent excessive degradation of the neurochemicals in the perfusate, the outflow channel of the fluid swivel should have a very low dead volume. To obtain enough torque to turn the fluid swivel it is necessary for the animal to be wearing some type of harness, which is attached to the fluid swivel by a sturdy cable. Prior to the experiment, the animal should have become accustomed to wearing the harness since stress (e.g., novel restraint) increases the catecholamine and indoleamine concentrations in the brain (Bliss et al., 1968; Weiss et al., 1981; Kelly and Franklin, 1984). We have found a fluid swivel to be essential since simply putting an animal, especially a rat, on a rotating floor does not work particularly well. As the floor is rotated to untwist the inflow and outflow the rat will run in the opposite direction at the same speed the floor is being rotated. The rat can do this with amazing accuracy.

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