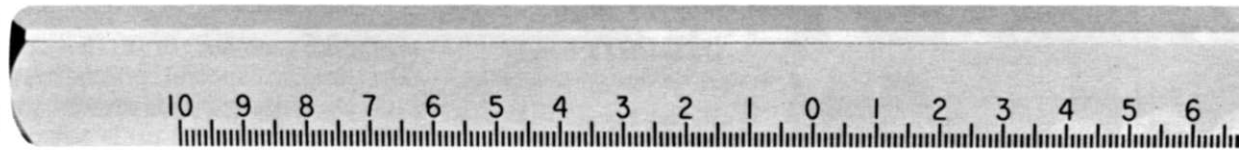
KOPF CARRIER

October, 1995

PUBLISHED BY DAVID KOPF INSTRUMENTS • TUJUNGA, CALIFORNIA • NO 44



The Loop Dialysis Catheter: A New Technology for Chronic Spinal Dialysis

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INTRODUCTION

An important experimental method for assessing the role of specific neurotransmitter systems is to establish the extravascular-extracellular levels of the agent in the vicinity of the terminals from which the hormone is believed to be released. Ex vivo methods, such as minces or slices have advantages in they permit exposure of specific brain sites. On the other hand such methods typically remove the connectivity essential for the interpretation of mechanism. The use of in vivo models, while inherently more complex have found favor as they permit assessment of the physiology of the intact system. Several approaches of measuring the extracellular levels have been developed. The use of voltametric electrodes may be of advantage, but they are limited in the nature of the product they can measure and they retain problems of selectivity. Accordingly, sampling of the extracellular space and assays of the perfusate have been the method of choice. In brain, local perfusion techniques, such as the push-pull cannula (Yaksh and Yamamura, 1974), or superfusion of portions of the cerebrospinal (Feldberg and Sherwood, 1954) or spinal (Yaksh, 1985) axis have been employed. Such superfusion techniques are limited in that they involve unpredictable perfusion pathways and de-pened upon unobstructed patency to avoid tissue injury. Accordingly, the application of dialysis membrane devices have found increasing favor. In brain such probes, typically concentric in nature have been described and widely employed (Benveniste et al., 1984).

In the past 15 years, there has been an increasing interest in the transmitter pharmacology of the spinal cord. Dialysis systems have been developed in which a concentric cannula is inserted into the tissue (Brodin et al, 1987) or a single fiber inserted transversally through the parenchyma (Skilling, et al, 1988; Sorkin, et al, 1988). These models while sampling the parenchyma are typically unstable and difficult to maintain for extended periods in the unanesthetized animal and require an extensive exposure after removing portions of the paravertebral musculature and a partial laminectomy. In recent work, we have developed an alternative model, the loop dialysis catheter that diminishes surgical exposure and permits extended dialysis sampling of the lumbar intrathecal space in the unanesthetized and relatively unrestrained rat for intervals in excess of 10 days (Marsala, Malmberg and Yaksh, 1995). In this article we describe the catheter and representative experiments emphasizing the utility of the preparation.

CONSTRUCTION

Intrathecal loop dialysis catheters are constructed from hollow fibers (300jam outer diameter, Filtral, AN 69-HF). The fibers (18 cm length) are coated with a thin layer of epoxy except for a 4 cm portion in the middle. Nichrome-Formvar wire (O.D.- 0.0026 ") is then passed through the fiber and both ends of the fiber are attached to pieces of silicone tubing (I.D.- 0.012"; 3.5 cm length). The dialysis fiber is then bent such that the uncoated portion, forms a "U"-shaped loop. Finally, both pieces of silicone tubing are bound together with silicone rubber at the level of their junction with the dialysis fiber (Fig.l). On occasion, it is desired to inject an agent intrathecally at a site adjacent to the dialysis loop. In this case, an 8.5 cm length of polyethylene tubing (O.D.- 0.012"; special

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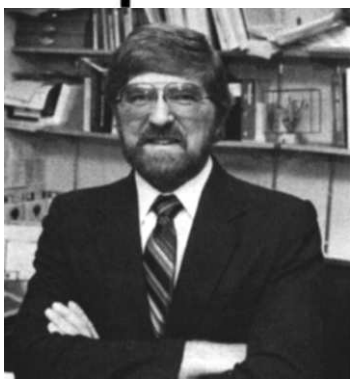

Editor's Column

Well, the big day is here, in fact, the BIG EVENT is over (just about 3 hours ago). No, I don't mean the World Series or the Super Bowl or even Christmas. I mean the Simpson Trial Outcome. This tells you when I am writing this, and I wonder if it will be one of those memories like the Kennedy assassination or the Challenger explosion that we will be able to ask "What were you doing when...?" I guess we need things like that once in awhile, since I can no longer ask students about Kennedy (they weren't born yet) and soon the Challenger event will be the same. I worry about having the money to pay a parking ticket; wouldn't it be nice to be able to drop $8 million on a defense.

The Society for Neuroscience Meetings are coming soon and this should be in your hands by then. Why not put a visit to the David Kopf Instruments booth on your agenda. I will be there a lot of the time and would like to chat about the Carrier or other issues with you. If you want to write an article for the Carrier, please see me there or leave me a message. There is a monetary reward for writing an article, and it is very widely distributed.

The article in this issue is very interesting, in that it reports a newly developed dialysis device for chronic use," amTon several of the uses to which it can be put. I am sure Dr. Marsala will be around the Society for Neuroscience meeting if you want to discuss it with him. Just leave a message at the Kopf Booth and we will be sure he gets it.

My wife and I are taking a trip to Australia during the last half of October (I am giving a talk) and are looking forward to it. I look forward to seeing you in San Diego, after our trip.

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purchase Clay Adams/ Becton Dickinson, NJ) is attached with a drop of superglue such that the tip is adjacent to one arm of the dialysis loop (Fig.l).

IMPLANTATION

To place the microdialysis catheter the rat is anesthetized with 2.5% halothane in a room air/ oxygen mixture (1:1) and the back of the head and neck shaved. The animal is then placed in a stereotaxic headholder with the head flexed forward. Anesthesia is maintained with 1.5% halo-thane delivered by mask. The back of the head is prepared with Betadine solution. A midline incision is made on the back of the neck. The muscle is freed at the attachment to the skull and retracted with a flat elevator, exposing the cisternal membrane. The membrane is opened with a 22 g needle and retracted with a small dural hook. The loop portion of the catheter is then inserted through the cisternal opening and passed caudal-ly 9 cm into the intrathecal space. This places the "uncoated" section of the dialysis loop at the T11-L5 spinal segments. Animals are typically allowed to recover for a minimum of 2-3 days prior to experimentation. Rats showing motor weakness or paresis upon recovery from anesthesia are euthanized.

PERFUSION, SAMPLE COLLECTION AND ANALYSIS

On the day of dialysis one of the externalized silicone tubes (Fig.l- Dialysis Inflow) was attached to a 30 cm length of PE-10 tubing (inflow) and the other arm to a 25 cm length of PE-10 (Fig.l-Dialysis outflow). A syringe pump (Harvard Compact Infusion Pump) was connected and the dialysis tubing perfused with artificial cerebrospinal fluid (ACSF) at a rate of 10 jal/ min. The ACSF contained (mM) 151.1 Na+, 2.6 K+, 0.9 Mg2+, 1.3 Ca2+, 122.7 C1-, 21.0 HCOs, 2.5 HPO4 and 3.5 dextrose. The ACSF is bubbled with 95% O2/5% CO2 before each experiment to adjust the final pH to 7.2. All experimental manipulations were typically preceded by a 30 min washout period and collection of 2 control samples (10 min each).

Dialysate samples were collected on ice and frozen at -20°C until analysis for amino acids and/or PGE2. Where both were assayed, samples were split prior to freezing.

Amino acids: Dialysis samples were routinely analyzed for a variety of amino acids. In the present studies, the glutamate and taurine levels are usually noted while in some experiments gly-cine and aspartate are reported. Analysis was accomplished by the use of the PITC (phenyl iso-thiocyanate) derivatization procedure using a Waters HPLC with a reverse phase CIS column (3.9 x 300 mm, 4um particle) and UV detector. Amino acid content was measured

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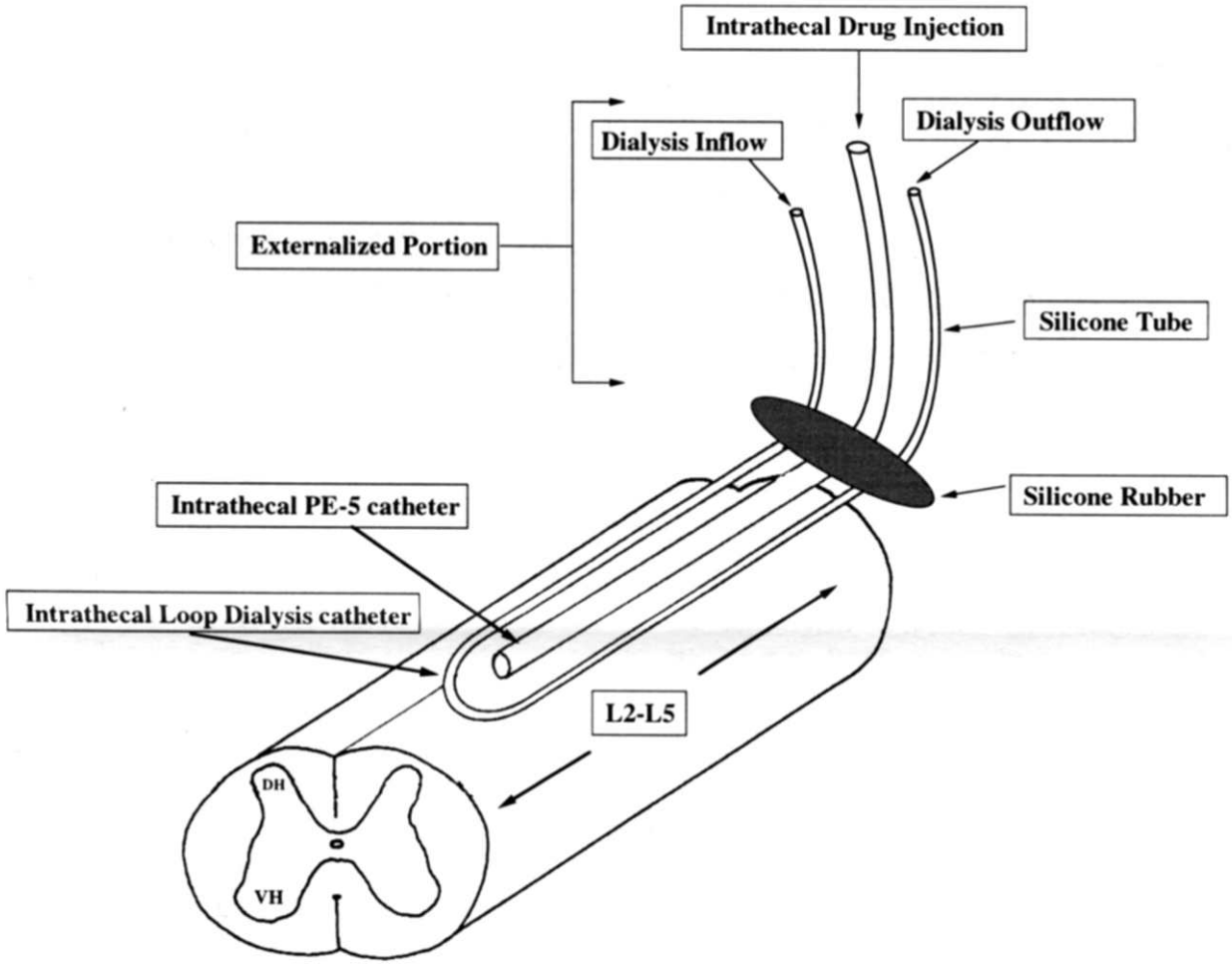

Figure 1. Schematic drawing of the loop-style intrathecal dialysis catheter and IT PE-5 catheter implanted into the intrathecal space.

from single 25 ul aliquots. Methionine sulphone was added to each amino acid sample and used as an internal standard. Sensitivity was 5-10 pmol/ injection. Amino acid peak heights were initially normalized to the methionine sulphone peak and then quantified based on a linear relationship between peak height and amounts of corresponding standards. External standards were run daily. All values are expressed as a percent of baseline.

Dialysate concentration of PGE2 was determined using a commercially available assay (Advanced Magnetics Inc., Cambridge, MA). The antibody is selective for PGE2 with less than 1.0 % cross-reactivity to PGFla, 6-keto PGFla, PGD2, or PG's of the A, B or D series, but cross-reacts with PGEi. Assay sensitivity is 2.8 fmol/sample.

Placement of the catheter in the hands of a trained operator requires approximately 20 min. In a prospective series of more then 500 rats, 35 rats required sacrifice due to motor dysfunction, while 22 rats were sacrificed because of occlusion of the dialysis catheter. The median study time in the prospective groups was 7 days.

STUDIES

1) Spinal cord Injury

To induce spinal compression in halothane anesthetized animals with previously implanted dialysis catheters, a 2F-Fogarty catheter was placed at the L1-L2 level in the spinal epidural space, through a partial laminectomy of the L5 vertebra. The balloon was inflated with saline for 60 s. After compression, animals recovered and survived for an additional 4 hr during which dia-lysate samples were collected and analyzed for aspartate, glutamate and PGE2. During the 4 hr postcompression period, motor and sensory functions were evaluated by assessing flaccidity/ spasticity and the response to hindpaw pinch. The placement of the epidural catheter had no effect on baseline concentrations of amino acids and PGE2. Spinal cord compression evoked significant increases in the concentration of aspar-tate (1242%; $p<0.01$) and glutamate (1036%; $p<0.01$) detected during the initial 10 min after compression, followed by a gradual decrease over the 4 hr post-compression period. However,

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both amino acids showed maintained significant increases at the end of 4 hr ($p<0.05$), (Fig. 2). Similarly, PGE2 levels increased immediately after compression (325%; $p=0.05$) with gradual normalization between 1-2 hr after trauma. Between 2-4 hr post-compression, a secondary increase occured when the concentrations reached even higher levels than those seen immediately after the compression (Fig. 2). Neurologically all animals displayed complete flaccid paralysis with loss of sensory function. This state remained unchanged for the full 4 hours.

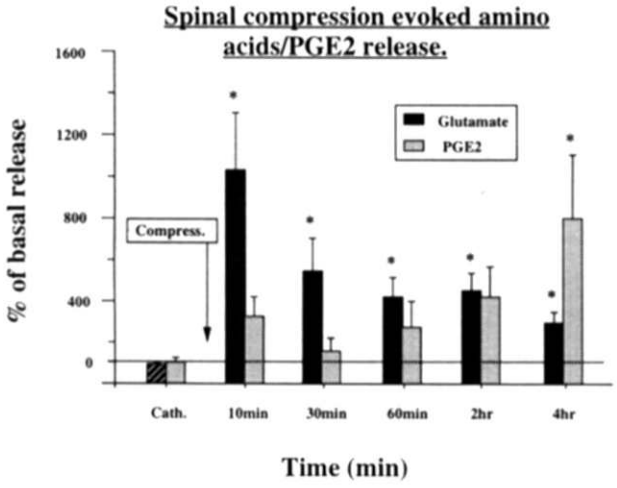

Figure 2. The effect of spinal cord compression on the spinal CSF glutamate and PGE2 levels for the period of 4 hrs after injury. Note a biphasic PGE2 release with the secod peak seen at 4 hrs (*$p<0.05$). (Adapted from Marsala, et al, 1995)

2) Systemic drug delivery and spinal CSF concentration To study the permeability of a drug through the blood brain barrier into the spinal extracellular space after systemic delivery, methylprednis-olone (MP) (20mg/kg) was injected intravenously (left jugular vein) during a 30 sec interval in halothane anesthetized rats (n=3). After injection spinal dialysate samples were collected in 10 min intervals for 2 hours. The concentration of MP in dialysate was determined by FID-gas chromatog-raphy by using dexamethasone as internal standard. After injection the concentrations of MP in the spinal CSF was as follows: (umol/ml): 10 min -<0.01; 20 min = 0.08±0.02; 30 min = 0.4±0.1; 40 min = 2.3±1.3; 50 min = 3.2±2.4; 60 min = 4±1.9; 70 min = 3.8±2.2; 80 min 4.1±2.5; 90 min = 3.9±2.8; 100 min = 3.2+1.7; 110 min = 2±1.8; 120min=1.8±1.6.

3) Peripheral inflammation and nociception To demonstrate the effects of peripheral noxious stimulation upon release of spinal transmitters, kaolin/carrageen an solution was injected into the knee joint and dialysate samples were periodically collected for the period of 24 hr in awake freely moving animals. Inflammation of the knee evoked a significant and prolonged glutamate release which persisted for at least 24 hr (Fig. 3A). This increase in the spinal CSF glutamate concentration correlated with the appearance of tactile (Fig. 3B) hypersensitivity which also persisted for at least 24 hr.

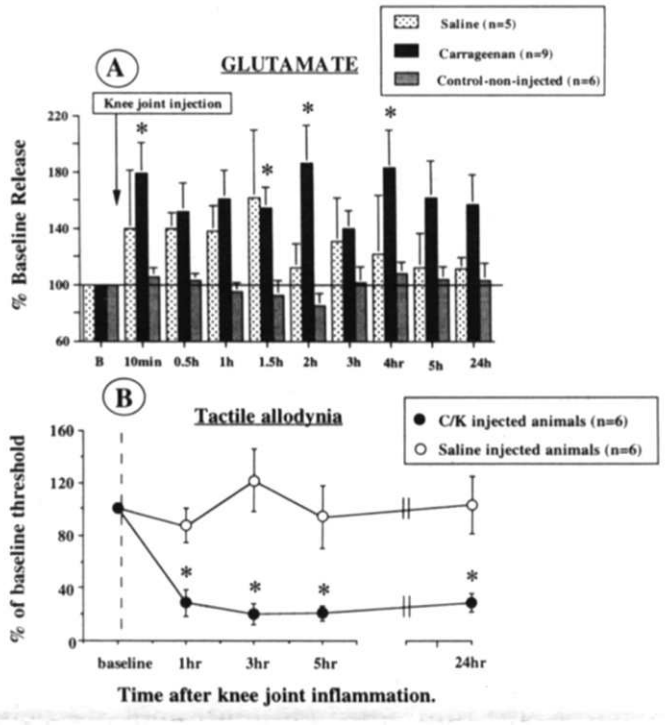

Figure 3. Time course of spinal glutamate release and the development of tactile allodynia after induction of knee joint inflammation. Note a prolonged release of glutamate and the presence of tactile allodynia for the period of 24 hrs (* $p<0.05$). (Yang and Marala, unpublished)

4) Intrathecal NMDA injection and spinal amino acid releaseTo demonstrate the spinal CSF levels of amino acids after intrathecal injection of NMDA, rats were implanted with intrathecal dialysis catheters and an additional intrathecal catheter for spinal drug delivery. Two days after implantation the animals were anesthetized with 2% halothane and maintained with 0.5-0.7% halothane. After baseline samples were collected, NMDA dissolved in saline was injected IT.

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Five sequential dialysate samples were collected at 8 min intervals. Injection of NMDA evoked a significant release of glutamate, taurine (Fig. 4) and aspartate with the peak seen 8 min after injection.

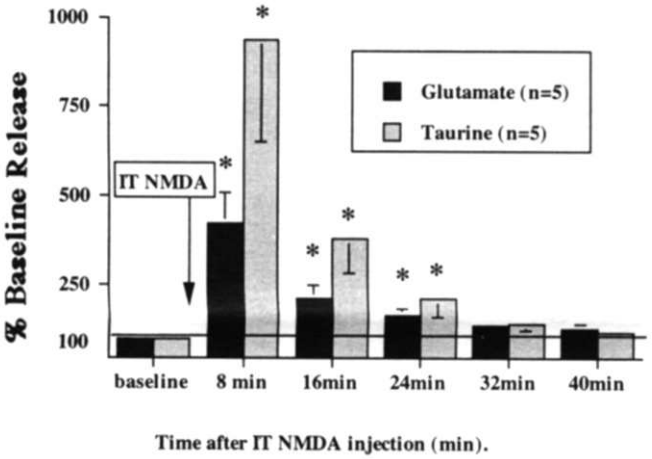

Figure 4. The effect of IT injection of NMDA (5ug) on spinal CSF glutamate and taurine concentrations. Significant increase in the concentra-tin of both neurotransmitters during the initial 24 min after injection was seen (*p<0.05).

COMMENT

These representative experiments demonstrate the potential utility of the ability to sample the lumbar intrathecal space of the unanesthetized rat. This sampling reveals that the extra-vascular extracellular levels of the endogenous transmitters vary in a predictable fashion with the activity of terminals from which they are thought to be released. Such measurements provide convergent data from pharmacological studies in which various antagonists have been delivered. Thus, with regard to the protracted afferent input, it has been shown that glutamate and prostaglandin levels rise and this elevation appears to be responsible in part for the allodynic state genarated by such protracted input (Malmberg and Yaksh, 1995). In addition to the measurement of the release of endogenous materials, the sampling procedure permits assessment of the movement of extraspinal agents in the spinal space (as with systemic or epidural delivery) or spinally administered agents from the intrathecal space. These techniques provide a strong tool for assessing the kinetics of spinal agents and for correlating these changes with behavior, e.g. analgesia as in the case of an opiate.

The model itself has particular advantage as it employs a relatively straight forward access with a method that is widely employed in the neuro-science community, e.g. the spinal catheter placement through the cisterna magna (Yaksh and Rudy,1976). Because of the minimal dissection, the recovery of the animal is accelerated and appears to represent a less traumatic intervention.

The majority of the techniques outlined above involving dialysis or perfusion require extensive surgical approaches including laminectomy and direct opening of the vertebral canal. These may significantly compromise the intactness of the system due to development of chronic inflammation in the areas of surgery/laminectomy and inflammation related ongoing low frequency noci-ceptive input. The present approach offers several advantages: 1) It completely eliminates the surgical dissection in the paravertebral lumbar area which eliminates the interaction between the surgical nociceptive inputs and nociceptive inputs arising from the hind limbs, an important site of stimulus application; 2) The implantation procedure is minimally invasive and of short duration, the placement of the catheter after anesthesia requires less than 15 min and immediately after implantation the majority of animals are ambulatory without signs of discomfort. The recovery rate is more then 90% success, as assessed by the recovery of motor and sensory function as well as by the patency of perfusion pathways; 3) The placement of the catheters in the intrathecal space via the cisternal route allows the catheters to exit the skin at a site which is relatively protected from attention by the animal. The result of this is that animals can be prepared with these catheters and experience long term (in excess of 10-15 days) patency, in contrast to parenchymal probes placed through a lumbar laminectomy; 4) Finally, the use of the loop design permits a highly flexible probe that results in minimal spinal damage. Moreover, the loop provides a large area of dialysis, enhancing local recovery.

A specific limitation of this spinal dialysis system is the inability to locate precisely the spinal terminals from which the release occurs. However, we believe that the advantages including preservation of tissue integrity and the ability to perform the perfusion concurrently with behavioral assessment in the unanesthetized rat outweigh this limitation for many studies.

Supported by NIH NS-32794

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