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Neurotransmitters Extraction by Local Intracerebral Dialysis in Anesthetized Rats

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HERNANDEZ, L., X. PAEZ AND C. HAMLIN. *Neurotransmitters extraction by local intracerebral dialysis in anesthetized rats*. PHARMACOL BIOCHEM BEHAV **18**(2) 159–162, 1983.—A new technique for in vivo neurotransmitter extraction was developed. Perfusion U shaped cannulae were chronically implanted in the caudate or the thalamus of rats. A segment of the cannula was a piece of dialysis tube of molecular weight cut off 1000. Measurable amounts of dopamine and norepinephrine were recovered from the perfusion fluid. Intraperitoneal amphetamine 10 mg/kg increased dopamine output from the caudate but not from the thalamus. The potential applications of this technique are discussed.

Dopamine Norepinephrine Intracerebral dialysis Amphetamine

THE physiological role of brain monoamines is not well known although they are possibly involved in many functions [7]. One of the reasons why such role is not clear is that most of the techniques for monoamine studies require to kill the animal as is the case with histochemical, brain homogenates, brain slices, or synaptosome analysis [2]. At best these techniques show a frozen picture of what was happening just before the animal died, and very often they do not allow to correlate a particular function with the variations of the extracellular flow of a monoamine.

A few in vivo techniques are currently available. Measurements of monoamines in cerebrospinal fluid have been done in primates and cats [1, 6, 11]. But this technique does not allow to determine the locus of production of the particular monoamine that supposedly is related with a specific function. Brain monoamine measurements have also been done in vivo with the push-pull cannula method [8,9]. Contrary to the cerebrospinal fluid technique, the push-pull cannula technique has the advantage of local extraction. But, although careful control of flow through the push-pull cannula prevents brain distention and minimizes clogging these troubles were associated with this technique [10]. Catecholamines also have been measured in vivo by electrochemical detection [3]. However with this method, the ascorbic acid present in the brain tissue interferes with dopamine measurements.

In the present paper we report a dialysis method that allowed to extract dopamine (DA) and norepinephrine (NE) and to monitor in vivo its regional extracellular flow without some of the inconveniences above mentioned.

METHOD

Cannula

The cannula was made of 26 and 33 ga stainless steel tubes, and hollow cellulose fibers of molecular weight cut off 1000 (Cole Parmer Instruments Company, Chicago, IL) (Fig. 1). Two pieces of 26 ga tubes 10 and 15 mm long were cut off (pieces A and E). A 15 mm long 33 ga tube (piece B) was U shaped at one of its ends. The straight end was inserted into tube A and soldered with stainless steel solder. The bent end was inserted into a 6 mm long piece of hollow cellulose fiber (piece C) and fixed with epoxy cement. Another 33 ga 10 mm long tube (piece D) was inserted and soldered in piece E, and inserted and fixed with epoxy in piece C. In order to facilitate insertion into the hollow fiber, pieces B and D were reduced by electrolysis [4]. After joining all the pieces, the two branches of the cannula were held together with acrylic cement (Fig. 2, top).

Subjects and Surgical Procedure

The subjects were 37 Wistar male albino rats weighing between 250 and 350 g. One cannula was stereotaxically implanted either in the caudate or the thalamus of each rat. With incisors bar 2.4 mm below ear bar stereotaxic coordinates for the head of the caudate were: 8 mm anterior to the ear bar, 3 mm lateral to the saggital suture and 8 mm ventral to the skull surface; for the thalamus: 4 mm anterior, 2.8 mm lateral and 8 mm ventral to the same reference points.

Perfusion

Perfusion experiments started after a recovery period of one week. The rats were anesthetized with ether. The branch A of the cannula was connected via a PE-10 polyethylene tube to a perfusion 5 ml syringe loaded with Krebs Ringer bicarbonate solution [12]. The plunger was pushed by a pump (Harvard Apparatus Co.). The rate of inflow was 10 μl/min and each perfusion lasted 50 minutes. Output liquid

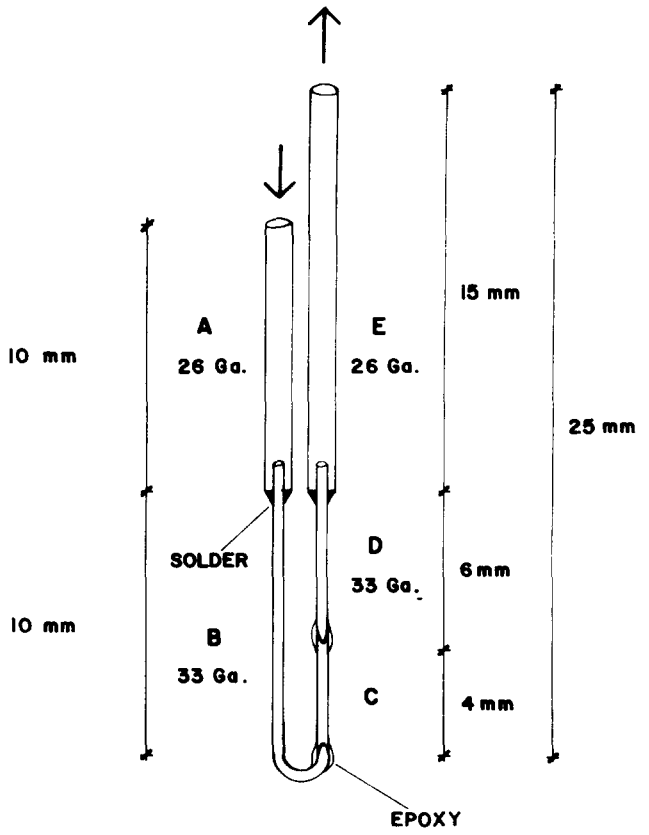

FIG. 1. Dialysis cannula. Tube C is the cellulose hollow fiber. The other tubes are stainless steel. Arrows indicate the direction of the perfusion fluid flow.

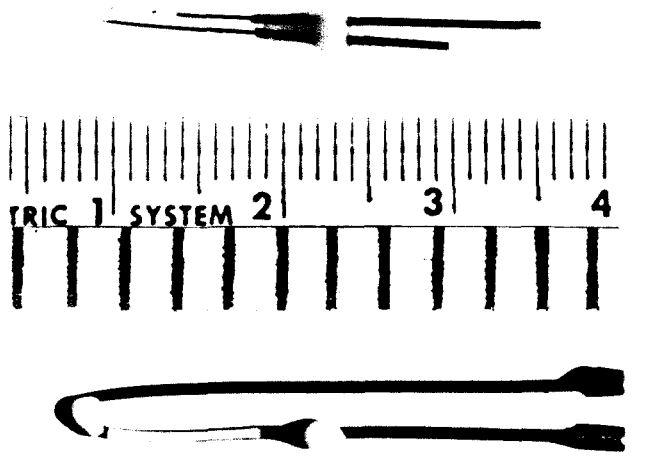

FIG. 2. Macrography of an actual dialysis cannula and a ruler (1 division=1 mm). Top shows the complete assembly with acrylic holding in place the branches of the cannula. Bottom shows the dialysis tube (between arrows) and epoxy cement connecting it to the stainless tubes.

emerging through branch E was collected via a PE-10 tube into an ice cooled plastic vial containing 0.5 ml of phosphate buffer of pH 6.5. At the end of the 50 minutes the volume of fluid left inside the syringe was measured as well as the volume collected into the vial. Although, these volumes were the same, transient changes of perfusion flow might have occurred because we did not measure flow continuously. In each experiment three 500 μl samples were taken. After the first sample, 50 min from the start of the experiment, the perfusion was stopped; and the animals received an intraperitoneal injection of either 10 mg/kg of amphetamine dissolved in 0.3 ml of saline or the same volume of saline. Perfusion was resumed 15 minutes later and the next two samples were obtained. Each rat was perfused on only one test day, but 2 or 3 weeks after the experiment cannula patency was verified.

The rats were killed with chloroform and perfused through the heart with formaline. The brain was dissected out, frozen and histologically sliced in order to localize the track of the cannula. Gliosis of the brain tissue was assessed by electron microscopy (E.M.) in three extra rats implanted with cannulae in the caudate. The brain tissue around the cannula was sliced and observed by E.M. at 3000×. The perfusion samples were analyzed by the fluorometric technique of Jackobowitz *et al.* [5]. Neurotransmitter flow was calculated dividing the total amount of neurotransmitter in the sample by 50 min. Statistical analyses were done by repeated measures analysis of variance, and Student's t test.

RESULTS

Pre-injection DA and NE flows were greater in the caudate nucleus than in the thalamus (Table 1) and DA flow was greater than NE flow in both the caudate and the thalamus. In the caudate the range of DA basal flow was from 0.016 ng/min to 0.273 ng/min. In the thalamus the range of DA basal flow was from levels not detected to 0.186 ng/min. In 6 out of the 15 perfusions of the thalamus it was not possible to detect DA at all. In saline injected rats DA flow in the caudate was significantly greater than NE flow, $F(1,14)=79.5$, $p<0.001$. DA and NE flows decreased after the first sample, $F(2,14)=11.91$, $p<0.001$, and there were also different flows for different rats, $F(7,14)=120.9$, $p<0.001$.

After the amphetamine injection DA flow increased in the caudate while it decreased after saline (Fig. 3), $F(1,10)=11.55$, $p<0.01$. With data expressed as percent of the first sample, the difference between DA flow after amphetamine vs saline was also significant, $F(1,10)=53.8$, $p<0.001$. Amphetamine or saline injections did not affect either DA and NE flows in the thalamus or NE flow in the caudate.

Histological analysis showed that the cannulae aimed to the thalamus were located in the posterior and dorsal nuclei of the thalamus. The cannulae aimed to the caudate nucleus were in the head of the caudate in 9 rats (Fig. 4), and only partially in the striatum in other 10 rats. E.M. studies showed a bicellular layer of glial cells around the cannulae, and the intracellular space looked normal as close as 10 micrometers from the cannula track.

DISCUSSION

The results show that intracerebral dialysis in vivo for

TABLE 1

BASAL FLOW OF DOPAMINE AND NOREPINEPHRINE IN THE CAUDATE AND THE THALAMUS

	Caudate (n=22)	Thalamus (n=15)	t*	p
Dopamine (ng/min)	0.115 ± 0.016	0.047 ± 0.015	2.84	<0.01
Norepinephrine (ng/min)	0.077 ± 0.015	0.026 ± 0.007	2.65	<0.01

Flows are expressed as mean ± standard error of the mean.
*Student's t-test.

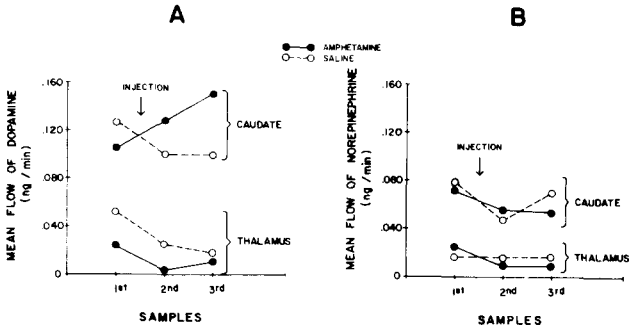

FIG. 3. Mean flow of dopamine (A) and norepinephrine (B) in the caudate (top) and the thalamus (bottom) before and after amphetamine or saline injection.

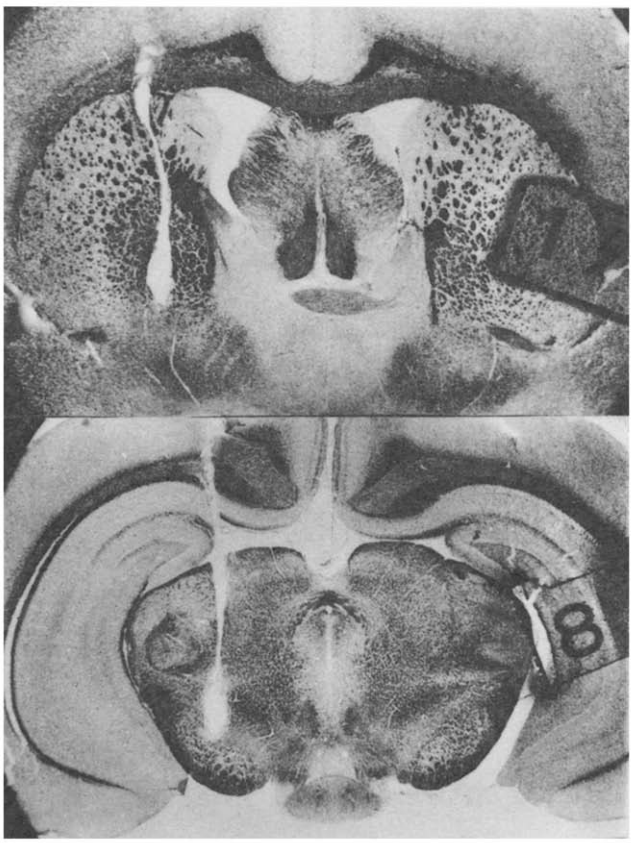

FIG. 4. Photomicrographs showing cannula in the caudate (top), and in the thalamus (bottom).

neurochemical studies is feasible. DA was recovered in all 22 caudate trials. DA was also recovered from the thalamus but only in 9 out of 15 perfusions and in lesser amounts. Therefore, the intracerebral dialysis can pick up the neurotransmitter from the region where it is known to be secreted. DA flow and NE flow decreased through the perfusion session. This could have been due to a slight depletion of neurotransmitter or to a dilution of the intercellular fluid because of dialysis. Amphetamine injections increased significantly the flow of DA in the caudate but did not change it in the thalamus. This pharmacological test shows that intracerebral dialysis permits to detect regional changes in the output of DA.

In 16 out of 53 rats perfusion experiments were impossible because implanted cannulae were found clogged 1 or 2 days after the implantation. The occurrence of this early clogging was reduced to 15 out of 55 rats in a new series of perfusions that we have done in freely moving rats. In this last experiment it was possible to detect changes in DA and NE flows during food deprivation (Páez and Hernández, in preparation). The main disadvantage of the dialysis cannula is its size, which is twice as great as the push-pull cannula. This prevents the use of dialysis cannula in small regions of the rat brain, i.e., hypothalamus. Nevertheless, the dialysis cannula might be used in the hypothalamus of species larger than the rat, such as primates. Alternatively, the dialysis cannula size could be reduced.

It should be possible to recover molecules of greater size than DA by changing the molecular weight cut off of the dialysis tubes.

The dialysis tube could also be useful for brain injections of drugs without causing brain distension. So, this technique should allow simultaneous injections of drugs and recovery of neurotransmitters in a small brain region.

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