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Measurement of somatostatin release in rat brain by microdialysis

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Abstract

We determined the most suitable conditions for measuring the somatostatin (SRIF) level by brain microdialysis and investigated its release from the hypothalamus. The relative recovery rate of SRIF was $8.4 \pm 0.5\%$ (mean $\pm$ SE) using a polycarbonate (PC) membrane with the push-pull method at a flow rate of 2 μl/min. Using tubes with an internal diameter of 0.28 mm and lengths of 5, 25, 50 and 100 cm, the relative recovery rates using a PC membrane with the push method were $8.2 \pm 0.5\%$, $7.3 \pm 0.6\%$, $6.2 \pm 0.5\%$ and $4.1 \pm 0.6\%$, respectively. When using tubes with an internal diameter of 0.1 mm and lengths of 5, 25, 50 and 100 cm, the relative recovery rates were $7.3 \pm 0.7\%$, $5.6 \pm 1.0\%$, $3.5 \pm 1.1\%$ and $1.4 \pm 0.7\%$, respectively. The relative recovery rate was $5.2 \pm 0.5\%$ with a polysulfone (PS-F, Fresenius) membrane, $4.5 \pm 0.4\%$ with a PS-H (Hospal) membrane, $2.6 \pm 0.2\%$ with an ethylenevinyl alcohol membrane (EVAL), $5.1 \pm 0.8\%$ with a polyvinyl alcohol (PVA) membrane and $10.4 \pm 0.8\%$ with a PS-K (Kaneka) membrane. With the push method, the extracellular SRIF level in rat pituitary was 42.8 ± 1.8 pg/ml with a PC membrane, 23.1 ± 2.9 pg/ml with an EVAL membrane at a flow rate of 2 μl/min. With the push-pull method, it was 52.7 ± 5.2 pg/ml using a PC membrane, 33.5 ± 2.8 pg/ml using a PVA membrane and 54.4 ± 3.2 pg/ml using a PS-K membrane. Intraperitoneal injection of urethane significantly increased SRIF from a basal level of 36.8 ± 10.6 pg/ml to 66.6 ± 12.5 pg/ml ($P < 0.05$) after 1 h; however, pentobarbital decreased it from 44.7 ± 4.1 pg/ml to 16.0 ± 4.6 pg/ml after 1 h. These data, which give optimum microdialysis conditions for measuring SRIF release in the rat, were useful for measuring the physiological release of SRIF from the hypothalamus.

Key words: Somatostatin; Microdialysis; Anterior pituitary gland; Pentobarbital; Urethane

1. Introduction

The push-pull perfusion method with a push-pull cannula has been used to obtain samples for measuring substances released in the brain under anesthetic-free, non-immobilized conditions (Kasting et al., 1981; Fukata et al., 1985). However, the problems with this method are that the pressurized stream of perfusate may seriously injure the tissue, and it is difficult to determine adequate push-pull conditions. To overcome these problems, Ungerstedt et al. (1982) developed a brain microdialysis method using a cannula the tip of which was sheathed with a dialysis membrane. This method was first applied to the in vivo analysis of monoamines, their metabolites and other classical transmitter substances of low molecular weight such as amino acids and acetylcholine. With the development of dialysis membranes and systems for sampling and analysis, brain dialysis has also been used in the determination of such neuropeptides as cholecystokinin (Hurd, 1989; Kato, 1989; DeMesquita et al., 1989; Takita et al., 1989), insulin-like growth factor I (Yamaguchi et al., 1991), neurokinin A (Lindefors et al., 1985, 1989b), neurotensin (Bean et al., 1989), opioids (Maidment et al., 1989), oxytocin (Kendrick et al., 1988a,b; Caldwell et al., 1989; Kendrick and Keverne, 1989; Hattori et al., 1990) and substance P (Brodin et al., 1983, 1987; Lindefors et al., 1985, 1986, 1987, 1989a,b; Morilak et al., 1988). However, because the recovery of these hormones and the sensitivity of the assay systems were unsatisfactory, the only hypophysiotropic hormone the release of which has been studied

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is luteinizing hormone-releasing hormone (Levine and Powell, 1989; Steele et al., 1992).

We examined the in vitro recoveries of SRIF using different perfusing methods with several outlet tubes and several different membranes. We optimized conditions to apply this method for monitoring the in vivo extracellular SRIF level in rat pituitary, and also studied the effects of anesthetics on extracellular SRIF levels in the pituitary.

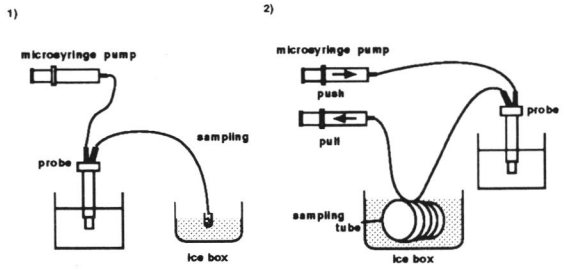

Fig. 1. Schematic diagram of perfusion systems. (1) push method; (2) push-pull method.

2. Materials and Methods

2.1. Recovery of SRIF in vitro

2.1.1. Influence of ultrafiltration on recovery of SRIF

A polycarbonate (PC) membrane (2 mm long; CMA 12, CMA Microdialysis, Sweden) was used as a microdialysis probe. Ringer's solution containing 0.1% bovine serum albumin (BSA) was perfused at a flow rate of 2 μl/min, using a pulse-free microinfusion pump (EP-60, Eicom, Kyoto, Japan) and 1- or 0.5-ml Hamilton gastight syringe. Tubes made of polyethylene (PE) or teflon, which were confirmed to absorb little SRIF (data not shown), were connected between the syringe and probe and between the probe and sampling tube. Perfusion was carried out using both the push-pull and push methods (Fig. 1) in order to assess the influence of internal pressure on microdialysis. For the push method, PE (internal diameter: 0.28 mm; lengths: 5, 25, 50 and 100 cm) and teflon tubes (internal diameter: 0.1 mm; lengths: 5, 25, 50 and 100 cm) were used as outlet tubes. The influence of perfusion rates on recovery was assessed by measuring relative recovery rates at perfusion rates of 1, 2 and 4 μl/min using the same probe together with outlet tubes with an internal diameter of 0.28 mm and a length of 50 cm.

For determining the relative recovery rate, 1 ng/ml SRIF was dissolved in Ringer's solution and kept at 37°C. A probe was inserted into this fluid, the dialysate was collected every hour, and its SRIF concentration measured by radioimmunoassay (RIA). The relative recovery rate was calculated from SRIF concentrations in the external and internal fluid.

2.1.2. Comparison of recoveries of SRIF with various dialysis membranes and plasma exchange membranes

Microdialysis probes with 2 mm membrane length were prepared using the following membranes (Table 1): polysulfone dialysis membranes (PS-F, Fresenius, Germany; and PS-H, Hospal, France), ethylenevinyl alcohol plasma exchange membranes (EVAL, Kuraray, Japan), plasma separation membranes made of polyvinyl alcohol (PVA, Kuraray, Japan) and plasma separation membrane made of polysulfone (PS-K, Kaneka, Japan). The relative in vitro recovery rates with these membranes were compared with that of the PC dialysis membrane in vitro. For PC, PS-F, PS-H and EVAL membranes, perfusion was performed by the push method at 2 μl/min flow rates using an outlet tube with an internal diameter of 0.28 mm and length of 50 cm. For PVA and PS-K membranes, perfusion was done by the push-pull method at flow rates of 2 μl/min. The relative recovery rate was determined as in the above experiment.

2.2. Experimental animal

Male Wistar rats, weighing 250–300 g, were individually housed in an air-conditioned room (22 ± 2°C)

Table 1
Characteristics of membranes used for microdialysis

Membrane (Supplier)	Polycarbonate (PC) (CMA microdialysis)	Polysulfone (PS-F) (Fresenius)	Polysulfone (PS-H) (Hospal)	Polysulfone (PS-K) (Kaneka)	Ethylenevinylalcohol (EVAL) (Kuraray)	Polyvinyl alcohol (PVA) (Kuraray)
Internal diameter	300	200	200	200	300	340
Thickness (μm)	100	40	40	50	100	50
Sieving coefficients						
β_2-microglobulin	– [a]	0.79	0.90	– [a]	– [a]	– [a]
Albumin	– [a]	< 0.02	0	0.8	1.0	1.0
MW cut-off (Da)	20,000	20,000	20,000	100,000	> 1,000,000	> 1,000,000

[a] not examined.

under artificial illumination (light, 9:00–21:00 h; dark, 21:00–9:00 h) and given regular rat chow and water ad libitum.

2.3. Experimental protocol

Rats were anesthetized by an intraperitoneal (i.p.) injection of 50 mg/kg pentobarbital. By using a brain-fixing device (David Kopf Instruments, USA), a guide cannula was implanted with its tip just dorsal to the anterior pituitary using the coordinates (0.9 mm lateral to the midline, 6.0 mm posterior to the bregma and 9.0 mm ventral to the dura), and fixed in position with dental resin (GC Reparisin, GC Shika, Kyoto). Cefotetan (20 mg/kg body weight (BW)) was injected after the operation to prevent infection.

One week after surgery, a PC, EVAL, PVA or PS-K membrane probe was inserted into each animal in an anesthetic-free, non-immobilized state. Experiments were started at 9:00 h, and perfusion was carried out by the push method with PC and EVAL membranes or by the push-pull method with PC, PVA and PS-K membranes at flow rates of 2 μl/min. At least 3 h after the start of perfusion, the dialysate was collected in ice-cooled test tubes every 30 min during 6 or 10 daytime hours for determination of SRIF levels.

The effects of anesthetics on SRIF levels were studied as follows. One week after surgery, a PC membrane probe was inserted into the animals in an anesthetic-free and non-immobilized state. Experiments were started at 20:00 h, and perfusion was carried out by the push method at flow rates of 2 μl/min. Fourteen hours after start of the perfusion, the animals were given i.p. injection of urethane ($n = 5$, 100 mg/100 g BW), pentobarbital ($n = 3$, 5 mg/100 g BW) or saline ($n = 3$, 0.1 ml/100 g BW). The dialysate was collected in ice-cooled test tubes every 60 min for determination of SRIF levels.

At the end of the experiment, the animals were deeply anesthetized with pentobarbital and fixed by the infusion of 10% formalin. The probe position was then verified histologically.

2.4. Determination of SRIF level in perfusate

SRIF levels were measured by RIA as reported previously (Saito et al., 1979; Saito, 1980). The lowest detectable SRIF level was 1.5 pg/tube, and a dose-response was observed in the 1.5–200 pg/tube range. The intra-assay coefficients of variation of 12.5, 25.0 and 50.0 pg/tube concentrations were 12.0%, 7.8% and 6.1%, respectively, while inter-assay coefficients of variation at the same concentrations were 13.4%, 7.2% and 8.3%, respectively. The cross-reactivity of SRIF(1–28)NH_2 with the SRIF antibody used in this study was 109.2 ± 4.5% (mean ± SD) on a molar basis (Hosoi et al., 1988).

2.5. Statistical analysis

Data are expressed as mean ± SE. The significance of differences between pairs of groups were tested by the unpaired Student's t test.

3. Results

3.1. Effect of ultrafiltration on recovery of SRIF

The relative recovery rate of SRIF with a PC membrane by the push-pull method at flow rates of 2 μl/min was 8.4 ± 0.5% (Fig. 2). Using PE tubes with an internal diameter of 0.28 mm and with lengths of 5, 25, 50 and 100 cm, the relative recovery rates using the push method were 8.2 ± 0.5%, 7.3 ± 0.6%, 6.2 ± 0.5% and 4.1 ± 0.6%, respectively. When using teflon tubes with an internal diameter of 0.1 mm and lengths of 5, 25, 50 and 100 cm, the relative recovery rates under the same conditions were 7.3 ± 0.7%, 5.6 ± 1.0%, 3.5 ± 1.1% and 1.4 ± 0.7%, respectively. Relative recovery rates were almost the same at 500 pg/ml and 10 ng/ml SRIF concentrations for the external fluid (data not shown).

The relative recovery rates at flow rates of 1, 2 and 4 μl/min using 50-cm-long PE with an internal diameter of 0.28 mm were 10.8 ± 0.6%, 6.2 ± 0.5% and 1.8 ± 0.1%, respectively (Fig. 3). The absolute recoveries were 6.5 ± 0.4 pg/h, 7.4 ± 0.6 pg/h and 4.4 ± 0.2 pg/h, respectively.

3.2. Recoveries of SRIF with various membranes

Using the push method at flow rates of 2 μl/min, relative recovery rates of SRIF were 5.2 ± 0.5% with a PS-F membrane, 4.5 ± 0.4% with a PS-H membrane and 2.6 ± 0.2% with an EVAL membrane. Using the push-pull method, relative recovery rates were 5.1 ± 0.8% with a PVA membrane and 10.4 ± 0.8% with a PS-K membrane (Table 2).

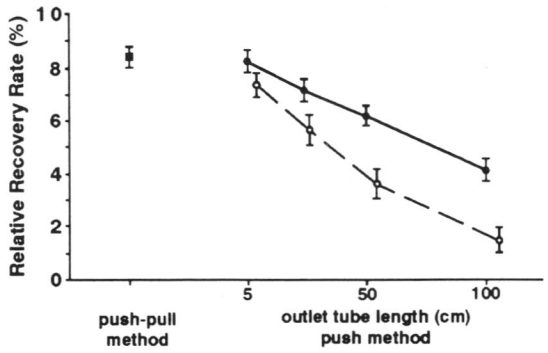

Fig. 2. Relative recovery rates ($n = 6$, mean ± SE) of SRIF with a PC membrane at a flow rate of 2 μl/min. Values were obtained by the push-pull method (■) and push method with outlet tubes measuring 5, 25, 50 and 100 cm long made of PE (●) and of teflon (○) tubing.

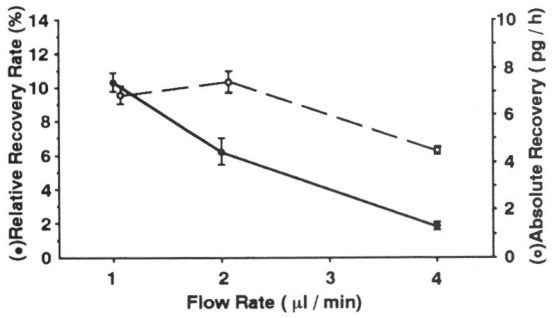

Fig. 3. Relative recovery rates ($n = 6$, mean ± SE) of SRIF at flow rates of 1, 2 and 4 μl/min using PE tubing 50 cm long with an internal diameter of 0.28 mm.

3.3. Extracellular SRIF level in rat pituitary in vivo

Using the push method at flow rates of 2 μl/min, extracellular SRIF levels in rat pituitary were 42.8 ± 1.8 pg/ml with a PC membrane and 23.1 ± 2.9 pg/ml with an EVAL membrane. By the push-pull method, SRIF levels were 52.7 ± 5.2 pg/ml with a PC membrane, 33.5 ± 2.8 pg/ml with a PVA membrane and 54.4 ± 3.2 pg/ml with a PS-K membrane (Table 2).

3.4. Effects of anesthetics on SRIF release

As shown in Fig. 4, SRIF levels increased significantly from a base level of 36.8 ± 10.6 pg/ml to 66.6 ± 12.5 pg/ml ($P < 0.05$) 1 h after i.p. administration of urethane. In contrast, it was reduced from 44.7 ± 4.1 pg/ml to 16.0 ± 4.6 pg/ml 1 h after administration of pentobarbital and did not change after administration of saline (from 38.0 ± 1.5 pg/ml initially to 36.6 ± 3.6 pg/ml after 1 h).

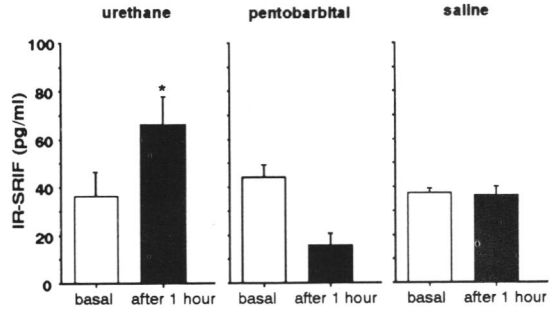

Fig. 4. Effects of anesthetics on the SRIF level (mean ± SE). Urethane, pentobarbital and saline were administered i.p. * $P < 0.05$ vs. basal level.

4. Discussion

Brain microdialysis has been used for studying neuropeptide secretion in the brain. However, investigation of the release of hypophysiotropic hormones using microdialysis is difficult because of the low recovery and the insensitivity of the available assays. Previous basic investigations on brain microdialysis have included examinations of dialysis membranes, perfusion rates (Kendrick, 1989) and pressure gradients, and also of the microdialysis apparatus (Ruggeri et al., 1990). However, since the influence of ultrafiltration on microdialysis had not been examined in detail, the optimal test conditions remained uncertain. We examined these factors, and succeeded in monitoring SRIF release from the hypothalamus using our technique.

Two major factors involved in microdialysis are ultrafiltration and diffusion. With regard to ultrafiltration, we found that by the push-pull method, where transmembrane pressures are less than in the push method, SRIF recovery was highest with a PC membrane (molecular weight (MW) cut-off 20,000 Da). Recovery rates increased with an increase in the internal diameter of the outlet tube and decreased with an increase in tube length. At high perfusion rates, the absolute recovery tended to decrease with a decreased internal diameter of the outlet tube. These data indicate that the choice of perfusion method and internal diameter and length of the outlet tube are important factors when measuring neuropeptides and hypothalamic hormones by brain microdialysis. Although the push method can be used with a simple circuit, some neuropeptides may be measured more effectively by the push-pull method. This can be accomplished by improving the circuit to reduce dead volume by reducing the internal diameter of the tube.

It is necessary to use an outlet tube of at least 40–50 cm in length when experiments are conducted for more than 24 h under anesthetic-free and immobilized con-

Table 2
Relative recovery rates and the SRIF level in vivo with different membranes

Membrane	PC		PS-F	PS-H	EVAL	PVA	PS-K
Perfusion	push	push-pull	push	push	push	push-pull	push-pull
Relative recovery rate in vitro (%, $n = 6$)	6.1 ± 0.5	8.4 ± 0.5	5.2 ± 0.5	4.5 ± 0.4	2.6 ± 0.2	5.1 ± 0.8	10.4 ± 0.8
SRIF level in vivo (pg/ml, $n = 3$)	42.8 ± 1.8	52.7 ± 5.2	–	–	23.1 ± 2.9	33.5 ± 2.8	54.4 ± 3.2

–, not examined; mean ± SE.

ditions. Based on our initial experiments, we used an outlet tube with an internal diameter of 0.28 mm and a length of 50 cm in the experiments of the push method at a flow rate of 2 μl/min.

In microdialysis, diffusion rates should be affected by the membrane composition, thickness and pore size. Kendrick et al. compared the percent recoveries of various peptides with a cellulose membrane, Amicon Vitafiber and PC membrane, and found that a PC membrane was the most effective (Kendrick, 1989). Yamaguchi et al. (1991) used an EVAL membrane for measuring brain IGF-1 levels. In the present study, we compared recovery rates for four synthetic membranes: PC, PS, EVAL and PVA membranes. The relative recovery rate by the push method was highest with a PC membrane, being comparable to that with plasma separation membranes with MW cut-offs of 100,000 Da or more. Since the MW of SRIF is 1638, in practice, membranes with a MW cut-off of 20,000 Da are effective. The relative recovery rate with a PS membrane (PS-F or PS-H) was slightly less than that with a PC membrane. However, since tissue damage increases with increased external diameter of the membrane, PS membranes could be used for microdialysis in smaller target tissues.

SRIF levels in rats were reported to be 0.108 μg/g protein in the hypothalamus and 0.036 μg/g protein in extrahypothalamic tissues (King and Millar, 1979). SRIF levels were reported to be 5.19 ng/mg protein in the paraventricular nucleus, 23.77 ng/mg protein in the arcuate nucleus, 2.85 ng/ml protein in the mediodorsal nucleus, 352.54 ng/mg protein in the median eminence and 6.4 ng/mg protein in the pituitary lobe (Palkovitis et al., 1980). SRIF concentrations in hypophyseal portal blood of rats anesthetized with ketamine were 112 ± 9 pg/ml (Plotsky and Vale, 1985). The SRIF level in the median eminence of rats with normal GH pulses varies between 10 and 100 pg/15 min, as determined by push-pull perfusion (Kasting et al., 1981). In rats with suppressed GH pulses, the level varies between 50 and 500 pg/15 min. Although the median eminence has been used in previous studies on SRIF release, in the present study we selected the anterior pituitary because it is easier to insert a probe into this region, and the operation does not damage the hypothalamic nuclei (Levine and Powell, 1989). Extracellular SRIF levels in the anterior pituitary were about 34.7–67.1 pg/ml when measured with a PC membrane by the push method. SRIF levels in vivo with different membranes paralleled the relative in vitro recovery rates of each membrane. These results confirm that PC and PS membranes are useful in studying the release of hypophysiotropic hormones by brain microdialysis.

The release of SRIF by the hypothalamus is reportedly stimulated by urethane and inhibited by pentobarbital (Chihara et al., 1979; Saito et al., 1979; Plotsky and Vale, 1985). These effects were confirmed in our study, which strongly suggested that brain microdialysis is a useful technique for studying the release of hypophysiotropic hormones including SRIF.

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