Histochemical Demonstration for the Release of Norepinephrine from the Sympathetic Nerves in the Cat Spleen

In the present communication the release of norepinephrine (NE) from the splenic nerves of cats has been demonstrated at a microscopic level by using a histochemical fluorescence procedure.

Methods and materials. The cat spleen was isolated in the abdomen and perfused at a rate of 7 ml/min with Krebs-bicarbonate solution at 33–35°C[1]. In some experiments spleens were perfused with Krebs-bicarbonate solution containing phentolamine (3 μg/ml). A small portion of the spleen was removed before nerve stimulation and left in the abdominal cavity. In order to demonstrate histologically the release of NE from sympathetic nerves, the arterial and the venous flows were stopped during the period of nerve stimulation (30/sec for 60 sec). As the stimulation continued and the flow had stopped, liquid nitrogen was poured directly into the abdominal cavity to freeze the spleen. The small portion of the spleen removed before stimulation was also simultaneously frozen, along with the stimulated portion of the spleen. Small blocks of the frozen tissue were then prepared for histochemical NE fluorescence studies[2].

Results. Figure 1 shows the photomicrographs of a phentolamine-treated spleen before and during nerve stimulation. Figure 1A shows discrete fluorescent fibers in 3 small arteries of the control unstimulated portion of the spleen. The fluorescence is located mostly in the adventitial layer. Internal elastic lamina is faintly visible in one of these arteries. Figure 1B shows development of diffused bright fluorescence over medial layer during nerve stimulation and flow-stop. Fluorescence is bright, but so diffuse that different regions of the arterial wall cannot be identified with certainty. A diffuse fluorescence is also seen around a trabeculum and a cluster of 3 small arteries. Figure 1C shows a transverse section of an artery, a nerve bundle and a vein during nerve stimulation and flow-stop; two small arteries are also seen. Discrete, localized fluorescence, so characteristically present in the adventitial layer of unstimulated splenic arteries, cannot be observed after stimulation and flow-stop. Qualitatively similar results were obtained from untreated spleens, but the intensity of fluorescence of stimulated portions of the spleen was weaker.

Discussion. Present experiments demonstrate the release of NE from post-ganglionic sympathetic nerves of the cat spleen with the help of a histological technique. This was possible only after diffusion of the released transmitter was restricted by flow-stop and the quantity of NE released by nerve stimulation was enhanced by the use of phentolamine. The fact that in a normal spleen the fluorescence is restricted only to the sympathetic nerve endings, whereas after nerve stimulation the fluorescence spreads over the smooth muscle cells of the medial layer and internal elastic lamina and becomes less intense, offers a direct evidence for the release of NE from sympathetic nerve endings.

Histochemical observations[3,4] show that smooth muscle cells, collagen, and elastic tissue of the isolated arteries, develop intense fluorescence after infusion of large quantities of NE. The fluorescence was not affected by 10^{-5} g/ml of phenoxybenzamine[4]. GILLESPIE et al.[2–4] showed that the threshold concentration of NE to make these tissues fluoresce was of the order of 10^{-5} g/ml. Since

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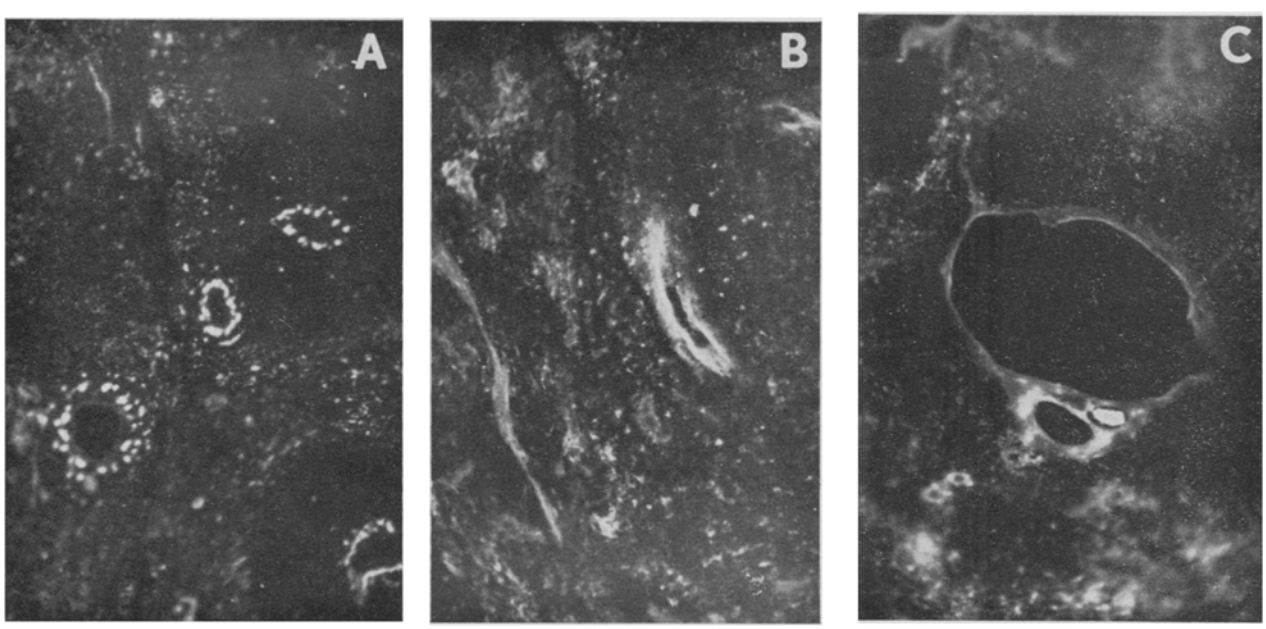

Figure A) shows a transverse section of an unstimulated portion of the spleen showing 3 blood vessels with the characteristic arrangement of fluorescent fibers in the adventitial layer. Figure B) shows the development of fluorescence in 1 artery and a strand of trabeculum during nerve stimulation and flow-stop. This section is tangentially cut. Figure C) is a transverse section and shows the effect of nerve stimulation and flow-stop on the development of fluorescence in an artery, nerve bundle, vein, and 2 small arteries. ×205.

the intensity of fluorescence of the tissues after nerve stimulation and flow-stop was obviously much greater than that produced by threshold concentrations of NE, we have to conclude that NE concentrations of several orders higher must have been present in the immediate vicinity of these smooth muscle cells and elastic tissue to make them brightly fluoresce. This would mean that even higher concentrations would be found in the immediate vicinity of the nerve ending after nerve stimulation. Even though flow was stopped during stimulation, some limited local diffusion down the steep concentration gradient, and hence dilution, must have occurred by the time the transmitter reached the smooth muscle cells. It is therefore conceivable that concentrations of NE greater than 10^{-5} g/ml may be found around the nerve terminals soon after stimulation.

Résumé. Le développement de fluorescence diffuse provoquée dans les cellules lisses, les trabécules et le tissue élastique par la stimulation des nerfs spléniques du chat pendant l'occlusion, est tenu pour évidence directe de la libération de noradrénaline provenant de nerfs sympathiques.

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Dentatectomy: Absence of Effects on Dyskinesias Arizing from Chemical Stimulation of the Striatum in Rats[1]

A useful experimental model of dyskinesias arizing from an induced excitatory-inhibitory imbalance in the striatum has been developed[2]. This model was used in the present report to evaluate the effects of dentatectomy on dyskinesias of striatal origin in awake and freely moving rats. The study was prompted by recent attempts to produce relief of parkinsonian dyskinesias by dentatectomy[3,4] and related attempts to alter lesion-induced tremors in monkeys[4]. In addition, COOPER[5] has suggested that the cerebellum plays an important role in the production of parkinsonian and related postural tremors.

Materials and methods. Stainless steel cannulae were permanently implanted in the neostriatum (nucleus caudatus-putamen) of young adult male Holtzman rats (250–300 g body wt.) according to the prodecure of DILL et al.[2]. The cannulae were placed stereotaxically at the level of the anterior commissure with coordinates obtained from the atlas of the rat brain, by PELLEGRINO and CUSHMAN[6]. Seven days after cannulation, each rat was injected intrastriatally (IS) with 1.5 µg of carbachol (Carcholine), 0.5 mg/ml saline. IS-injections were made at a rate of 1 µl/min to a total of 3 µl. The details of the injection technique were described previously by DILL et al. in 1966[2].

The dyskinesias resulting from the IS injection of carbachol were ranked according to the following system:

Type of dyskinesia	Rank of dyskinesia						
	0	1	2	3	4	5	6
Contralateral forelimb tremor		[a]	[b]	[b]	[c]	[c]	[c]
Facial tremor			[a]	[a]	[b]	[c]	[c]
Chewing motions			[a]	[a]	[b]	[c]	[c]
Neck tremor				[a]	[a]	[b]	[c]
Contralateral hindlimb tremor				[a]	[b]	[b]	[b]
Hyperextension of trunk					[a]	[b]	[b]
Bilateral forelimb tremor					[a]	[b]	[b]
Sialorrhea						[a]	[b]
Generalized convulsions							[a]

[a] Slight intensity and brief duration (1–5 min). [b] Moderate intensity and duration (6–40 min). [c] Severe intensity and long duration (more than 40 min).

The frequency of the contralateral forelimb tremor was measured by means of a magnetic tremor recorder[7]. Latency of effect was expressed as the time interval between initiation of the IS injection and the first appearance of tremor.

Two days after the initial carbachol injection, each rat was lesioned stereotaxically in the dentate nucleus[6] by means of a LM-4 Grass lesion maker. Three groups of 5 to 7 rats were lesioned in the dentate nucleus according to the following plan: Group 1, ipsilateral to the cannula site; Group 2, contralateral to the cannula site; and Group 3, bilaterally.

[1] This work was supported by Public Health Service Research Grant No. NS07739-03, from the National Institutes of Neurological Diseases and Stroke.

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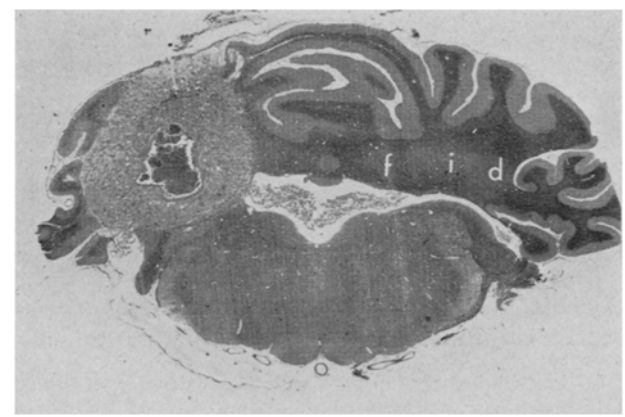

Large unilateral lesion of rat cerebellar deep nuclei on left. Intact nuclei labeled on right: f, fastigial nucleus; i, interposed nucleus; d, dentate nucleus. ×7.