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UPTAKE OF NORADRENALINE BY THE ADRENERGIC FIBERS OF THE SUBMAXILLARY AND SUBLINGUAL GLANDS OF THE RAT*

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Abstract—The uptake of noradrenaline by the submaxillary and sublingual glands of the rat was investigated histochemically and quantitatively. The administration of noradrenaline increased the noradrenaline fluorescence surrounding the serous acini of the submaxillary gland. The fluorescence in the walls of arterioles of both the submaxillary and sublingual glands was also intensified. On the denervated side, practically no fluorescence was seen after the injection of noradrenaline, although the quantitative determinations revealed a slight increase in noradrenaline levels. After the administration of noradrenaline to the animals treated with monoamine oxidase inhibitor, definite fluorescent fibers appeared among the mucous cells of the sublingual gland, where otherwise little fluorescence was seen. Such fluorescent fibers were never obtained on the denervated side. It was concluded that the mucous acini of the sublingual gland are innervated by adrenergic fibers, but normally the amount of noradrenaline in the fibers is too small or its distribution is too diffuse for it to be detected histochemically. A distinction between histochemical and biochemical findings is discussed.

IN RECENT papers,[1, 2] the authors have described the cellular localization of noradrenaline in the salivary glands of rat, by means of the histochemical formaldehyde condensation technique developed by Falck.[3] An interesting finding was that the fluorescence of noradrenaline is seen around the serous acinar cells of the submaxillary and parotid glands but not the mucous cells of the sublingual gland. Similar results were reported by other investigators.[4, 5] Noradrenaline fluorescence was conceivably undetected around the acinar cells of sublingual gland because (i) there is no adrenergic innervation to the cells, or (ii) adrenergic innervation exists, but the amount of noradrenaline in the fibers is too small or the distribution is too diffuse for it to be detected histochemically.

Previous reports from many laboratories have shown that catecholamines accumulate in adrenergically innervated tissues.[6–13] The purpose of these experiments is to show the existence of the adrenergic innervation on the acinar cells of sublingual gland of rat, which is demonstrable after the administration of noradrenaline.

MATERIALS AND METHODS

Experimental animals and procedures

Adult male Wistar rats weighing 200–250 g were used. The animals were given 200 μg/kg noradrenaline i.v. and killed by decapitation 2·5 min, or 10 min, or 2 hr

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after the end of injection. The submaxillary and sublingual glands were then immediately removed from both sides for histochemical examination or biochemical determination of noradrenaline. The high dose of noradrenaline was given to obtain optimal conditions for the histochemical demonstration. In some cases, DL-phenylisopropylhydrazine (JB–516) in a dose of 10 mg/kg was administered i.p. 24 hr before killing. In the chronic denervation experiments, the unilateral superior cervical ganglion was removed under sodium pentobarbital anesthesia, 40 mg/kg i.p., 9 to 10 days before sacrifice, and the contralateral innervated side was referred to controls.

Histochemical procedure

Pieces to be studied were frozen in a mixture of acetone and dry ice. After freeze-drying at −30° to −35° for 8–10 days, the preparations were treated with formaldehyde gas at 80° for 1 hr, according to Falck.[3] The preparations were then infiltrated *in vacuo* with paraffin at 60° for 10 min. Sections (3–4 μ) were placed on nonfluorescent slides and deparaffinized by the careful addition of xylene to the slides.

Fluorescence microscopy was performed with a Zeiss fluorescence microscope. The exciting light was provided by an Osram HBO 200 high-pressure mercury lamp and filtered through a 3-mm or 5-mm Schott BG 12 filter. The secondary filter was a Zeiss "50" filter. Usually a dark-field condenser for immersion oil was used for examination and photography of the section. Microphotographs were taken with Kodak Tri-X film. Exposure time ranged from 90 to 120 sec. The term "noradrenaline fluorescence" used here refers to a green fluorescence.

Biochemical determination

Quantitative determination of noradrenaline was carried out by the trihydroxyindole method described by Matsuo[14] of this laboratory. The salivary glands were pooled from at least three animals treated in an identical manner, blotted with filter paper, weighed, minced, and homogenized in ice-cold 0·4 N perchloric acid. After thawing, the homogenate was centrifuged at 10,000 *g* for 10 min. The supernatant was adjusted to pH 4·0 with 5 N potassium carbonate. The precipitated potassium perchlorate was filtered off by aspiration. According to Bertler *et al.*[15], noradrenaline in the filtrate was adsorbed on a Dowex 50 W–X2 column, then eluted with 8 ml of 1 N hydrochloric acid. The eluate was adjusted to 10 ml with distilled water. The oxidation of noradrenaline was performed by ferricyanide as described by Euler and Floding.[16] The Aminco-Bowman spectrophotofluorometer was used to measure fluorescence.

RESULTS

Treatment of freeze-dried tissues with formaldehyde gas readily converts primary catecholamines of the adrenergic fibers to 3,4-dihydroisoquinolines which show an intense green to yellow-green fluorescence. The specific noradrenaline fluorescence surrounding the secretory acini and granular tubules of the submaxillary gland increased in intensity after the injection of noradrenaline (Fig. 1a). The fluorescence of adrenergic fibers on the outside of the smooth-muscle layer in arteries of various sizes was also markedly increased in intensity. However, specific fluorescence was never seen in the acinar cells nor in the vicinity of the large excretory ducts which in the untreated glands showed no fluorescence. In the sublingual gland, the intensity

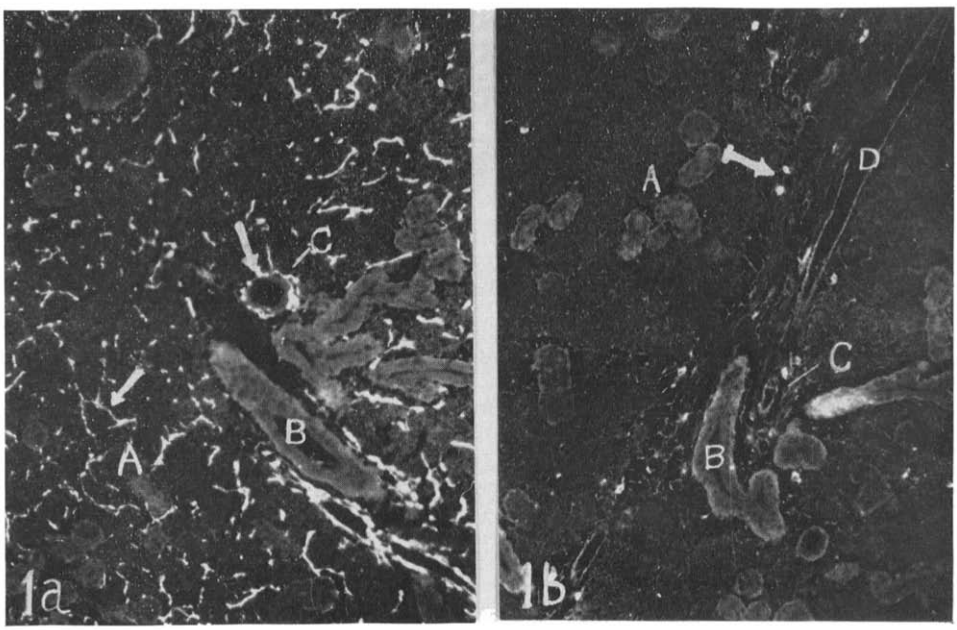

FIG. 1. Fluorescence microphotographs of freeze-dried sections treated with paraformaldehyde at 80° for 1 hr; A, acini; B, excretory duct; C, arteriole; D, venule (all figures). Submaxillary gland. Noradrenaline, 200 μg/kg i.v. at 2 hr. 1a: Innervated side. Intense fluorescence (at arrows) surrounding the acini and the arterioles. 1b: Denervated side. Practically no fluorescene of noradrenaline. Yellowish serotonin fluorescence of mast cells (at arrow); × 100.

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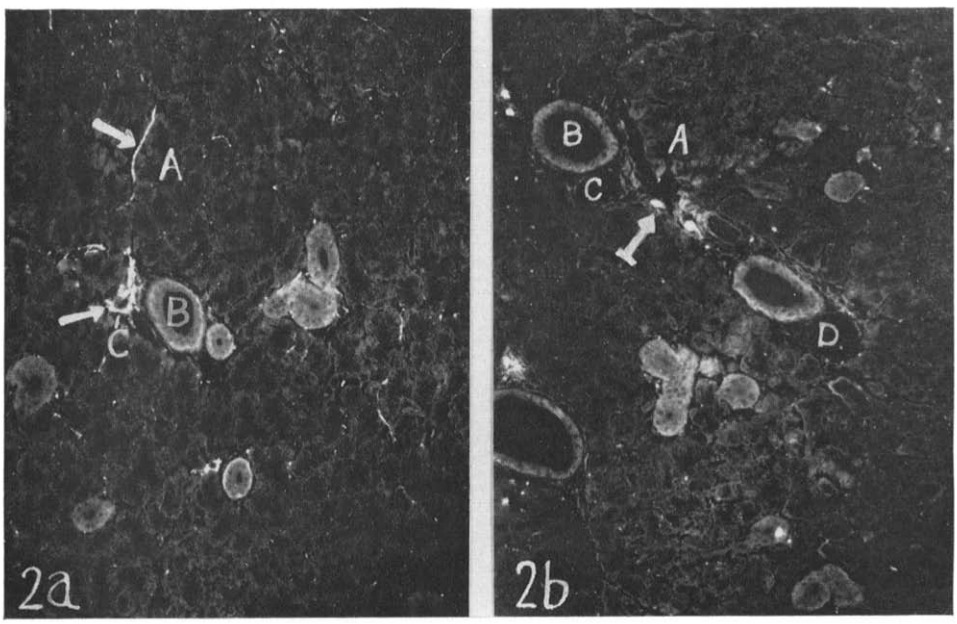

FIG. 2. Sublingual gland. Noradrenaline. 200 μg/kg i.v. at 2 hr. 2a: Innervated side. Occasional noradrenaline fluorescence (arrow) among the acini. Intense fluorescence around the arterioles (arrow). 2b: Denervated side. Practically no fluorescence of noradrenaline. Yellowish serotonin fluorescence of mast cells (arrow); × 100.

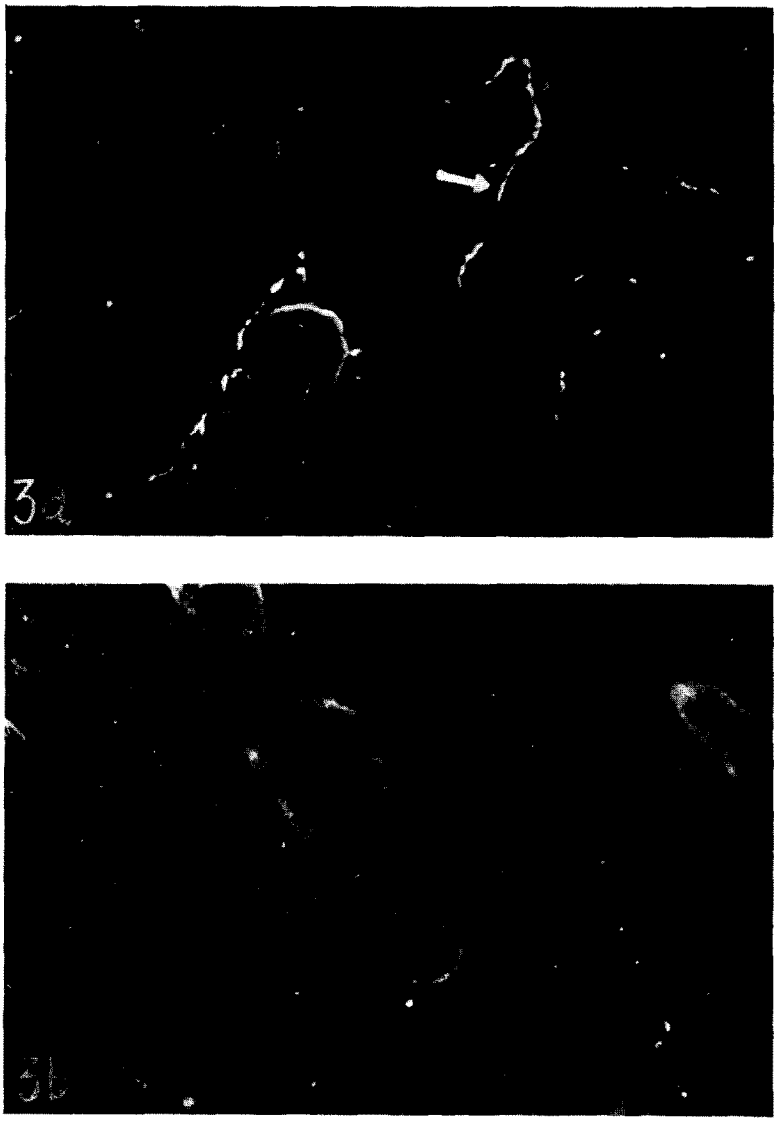

FIG. 3. Sublingual gland. JB-516, 10 mg/kg i.p. at 24 hr and noradrenaline 200 μg/kg i.v. at 2 hr. 3a: Innervated side. Definite noradrenaline fluorescence (arrow) surrounding the acini. 3b: Denervated side; no fluorescence; × 240.

of noradrenaline fluorescence increased in the walls of arterioles, the only place where the adrenergic fibers are normally detectable. Occasionally a slight fluorescence was seen among the acini (Fig. 2a).

As described in the previous report,[2] chronic sympathetic denervation resulted in a complete disappearance of specific noradrenaline fluorescence at all sites in the salivary glands. Only a yellow fluorescence of serotonin was seen near the blood vessels. The cells storing serotonin were identified as mast cells by metachromasia of toluidine blue. Injection of noradrenaline did not cause a reappearance of specific fluorescence in the denervated glands 2·5 min or 2 hr later (Figs. 1b and 2b). Although intense autofluorescence was seen, especially in the elastica interna of the arterioles, this was easily distinguishable from specific noradrenaline fluorescence, according to the histochemical and pharmacological criteria.[3]

The injection of noradrenaline into animals pretreated with JB–516 also increased the specific fluorescence in the salivary glands, but the intensity of fluorescence was no more than that after the injection of noradrenaline into untreated animals. A remarkable finding was that a fluorescence of about the same intensity as in the terminals of the adrenergic nerve fibers, located periacinary in the submaxillary gland, was developed at the periacinar sites of the sublingual gland, which in the normal animals showed no fluorescence (Fig. 3a). However, such fluorescence in the periacinar site was never obtained on the contralateral denervated side (Fig. 3b).

Parallel biochemical analyses confirmed that the noradrenaline levels in the salivary glands of normal and JB–516-treated animals increased by 70 to 90 per cent of the control at 2·5 min after the administration of noradrenaline, and the levels remained increased, if to a lesser extent, 2 hr later. After the injection, the denervated glands of untreated as well as JB–516-treated animals also took up (but in a small amount) noradrenaline, apparently without any return of specific fluorescence on the slide (Table 1).

DISCUSSION

Previous reports from this laboratory[1, 2] confirmed that the fluorescence of the rat salivary glands developed by paraformaldehyde treatment represented endogenous noradrenaline. Results obtained here show that the intensity of fluorescence increases after the injection of noradrenaline. Biochemical determinations also confirm the increases of noradrenaline level in the glands. Thus there is little doubt that the fluorescence which increased after noradrenaline was due to the injected amine itself. Previous reports from many laboratories have shown that catecholamines accumulate in adrenergically innervated tissues.[6–13] On the basis of these findings and from autoradiographic studies with tritiated noradrenaline,[8, 10] it has been postulated that the nerve endings of the sympathetic postganglionic system are the major site for noradrenaline storage. Histochemical demonstration of catecholamine uptake to the adrenergic fibers was performed by experiments *in vivo* and *in vitro* on rat iris.[17, 18] Our results also provide a direct histochemical demonstration of noradrenaline uptake by the adrenergic fibers in the salivary glands of rat. The fact that, even if noradrenaline was given to untreated or to JB–516-treated animals, no fluorescence developed in the acinar cells or in the vicinity of the large excretory ducts of the submaxillary gland where normally any specific fluorescence was undetectable, might suggest that noradrenaline is taken up only by the adrenergic fibers.

In the sublingual gland, the injection of noradrenaline after JB–516 treatment developed a new specific fluorescence at a site where otherwise little fluorescence appeared, and the fluorescence was abolished by chronic sympathetic denervation. In the previous reports,[1, 2] it was demonstrated in the rat sublingual gland that noradrenaline fluorescence was present only in the walls of arterioles, and that there

TABLE 1. NORADRENALINE LEVEL IN SALIVARY GLANDS OF RAT*

	Procedure	Time after injection	Noradrenaline (μg/g) Submaxillary gland	Noradrenaline (μg/g) Sublingual gland
Innervated	No drugs		1·25 ± 0·09 n = 9	0·32 ± 0·03 n = 9
	Noradrenaline	2·5 min	2·23 (1·77 – 2·73) n = 4	0·60 (0·57 – 0·64) n = 4
		2·0 hr	1·65 (1·37 – 1·82) n = 3	0·42 (0·30 – 0·56) n = 3
	JB–516, Noradrenaline	2·5 min	2·31 ± 0·25 n = 5	0·54 ± 0·05 n = 5
		2·0 hr	1·59 (1·22 – 1·97) n = 3	0·57 (0·26 – 0·92) n = 3
Denervated	No drugs		0·06 ± 0·01 n = 8	0·10 ± 0·03 n = 6
	Noradrenaline	2·5 min	0·10 ± 0·01 n = 6	0·24 (0·20 – 0·32) n = 3
		2·0 hr	0·08 (0·06 – 0·09) n = 4	0·22 (0·15 – 0·25) n = 4
	JB–516, Noradrenaline	10·0 min	0·08 (0·06, 0·09) n = 2	0·21 (0·11, 0·31) n = 2
		2·0 hr	0·09 (0·07 – 0·10) n = 3	0·18 (0·14 – 0·22) n = 3

* Mean ± S.E., or range (in parentheses); n = number of observations. Each value pooled from at least three animals. Denervation was made 9 to 10 days before killing. JB–516 was injected 24 hr before killing.

was no fluorescence in the vicinity of the secretory acini and excretory ducts. Similar findings were reported by other investigators.[4, 5] On the basis of these findings and from the cytological distribution of acetylcholinesterase, it was suggested that the secretory cells of the rat sublingual gland receive only a cholinergic supply and are devoid of adrenergic innervation. From the results obtained here, it is possible to postulate that the adrenergic fibers of the sublingual gland contain such low concentrations of catecholamines that few fluorescent fibers are detectable if the injection of noradrenaline is made to animals treated with monoamine oxidase inhibitor. Other studies, which will be reported elsewhere,[19] indicate that monoamine oxidase in the sublingual gland is in some degree associated with nervous tissues. It is considered that exogenous noradrenaline, which is otherwise readily attacked by monoamine oxidase of the gland, can be retained unchanged in the cytoplasm of nerve tissue after JB–516 treatment.

Although the histochemical fluorescence technique now available permits a direct demonstration of biogenic monoamines at cellular level, the lack of detectable fluorescence should not always be considered proof of the absence of the amines. It is possible that noradrenaline is too diffuse to be detected by fluorescence microscopy.

Accordingly, carefully controlled studies of treatment with a monoamine oxidase inhibitor and a precursor must be carried out before firm conclusions are drawn regarding the lack of adrenergic innervation in certain structures. In fact, some portion of noradrenaline remained resistant to chronic sympathetic denervation, and there was an increase, if in a small amount, of noradrenaline after the injection of this amine to the denervated animals, whereas any fluorescence was undected by the histochemical technique in either case. Such a distinction between histochemical and biochemical findings also might be explained by the aforementioned possibility of diffuse distribution of noradrenaline. Further studies are in progress to determine the subcellular localization of the amine under those circumstances.

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