ADRENERGIC COMPONENT IN THE MECHANISM OF ACTION OF SODIUM HYDROXYBUTYRATE

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Using the isolated rat vas deferens as the model the effects of sodium hydroxybutyrate on reserves of adrenergic mediator in nerve fibers and synaptic vesicles and the uptake and accumulation of exogenous noradrenalin were studied. It was shown by spectrofluorometric, fluorescence-histochemical, and cytochemical electron-microscopic methods that sodium hydroxybutyrate does not affect the reserves of adrenergic mediator but can block the uptake and accumulation of exogenous noradrenalin.

KEY WORDS: sodium hydroxybutyrate; reserves of noradrenalin; uptake of noradrenalin.

The effect of sodium hydroxybutyrate on the monoamine content in the brain has been investigated [4, 6, 8]. In particular, the results have shown that this compound increases the dopamine concentration but does not change the noradrenalin or serotonin concentrations [4, 5]. However, information on the effect of sodium hydroxybutyrate on the uptake and accumulation of exogenous noradrenalin (NA) by sympathetic nerve fibers is lacking. The effect of the compound on the penetration of exogenous NA into synaptic vesicles and its accumulation there likewise has not been investigated.

In the investigation described below these problems were studied by the use of the isolated rat vas deferens, richly innervated with sympathetic fibers [2, 8], as the model.

EXPERIMENTAL METHOD

The isolated rat vas deferens was divided into three parts and incubated in aerated Krebs's solution at 32°C for 1.5 h. The NA content was determined spectrofluorometrically in one part, the localization of the mediator was determined in the nerve fibers in another part fluorescence-histochemically [3], and the reserves of catecholamines in the synaptic vesicles were studied in the third part by a cytochemical electron-microscopic method [9]. The number of granular synaptic vesicles per square micron of section through the fiber was counted, using not less than 30-40 electron micrographs of each specimen for this purpose. The effect of sodium hydroxybutyrate on the NA content was studied 30 min after addition of the compound in a concentration of 0.1 EC_{50} (0.1 of the half-effective concentration), calculated for the effect of inhibition of contractions of the vas deferens in response to transmural electrical stimulation of postganglionic sympathetic nerve fibers [1].

EXPERIMENTAL RESULTS AND DISCUSSION

The results of the investigation of the effect of sodium hydroxybutyrates on reserves of adrenergic mediator in the tissues, nerve fibers, and synaptic vesicles are given in

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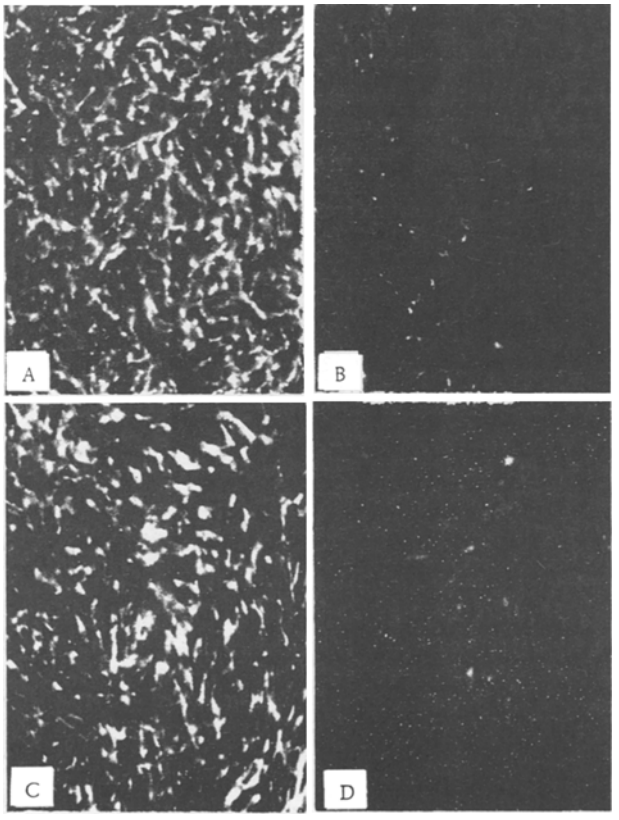

Fig. 1

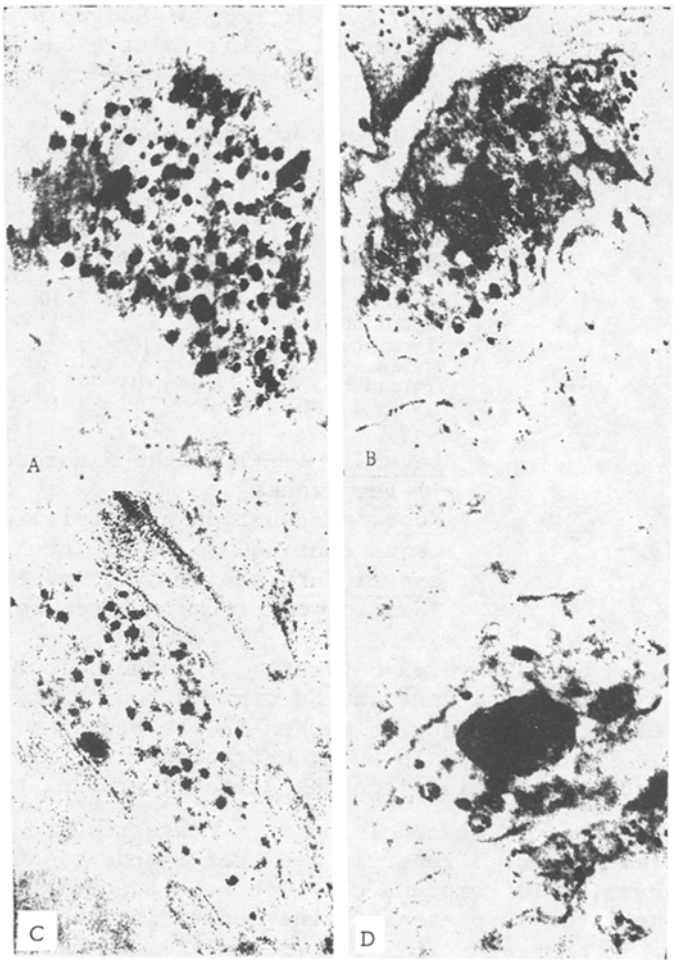

Fig. 2

Fig. 1. Fluorescence-histochemical detection of adrenergic mediator in sympathetic nerve fibers of rat vas deferens: A) fluorescence of adrenergic nerve fibers in control (160 ×); B) disappearance of reserves of mediator after incubation with tyramine (0.2 mM, 2 h; 160 ×); C) after incubation with tyramine followed by addition of noradrenalin ($3 \cdot 10^{-6}$ M) to the medium (200 ×); D) after incubation with tyramine and addition of sodium hydroxybutyrate ($4.4 \cdot 10^{-3}$ M) before noradrenalin (200 ×).

Fig. 2. Electron-microscopic demonstration of mediator in synaptic vesicles of adrenergic nerve fibers of rat vas deferens: A) control (33,000 ×); B) disappearance of reserves of mediator after incubation with tyramine (0.2 mM, 2 h; 25,000 ×); C) after incubation with tyramine and subsequent addition of noradrenalin ($3 \cdot 10^{-6}$ M) to the medium (28,000 ×); D) after incubation with tyramine and addition of sodium hydroxybutyrate ($4.4 \cdot 10^{-3}$ M) before noradrenalin (34,000 ×).

Table 1. In the control, adrenergic mediator was found in sympathetic nerve fibers forming a dense plexus of axon terminals (Fig. 1A). Synaptic vesicles 600-800 Å in diameter (less frequently 1200-2500 Å), containing catecholamines detectable as granules (Fig. 2A), could be seen in the adrenergic fibers. The number of granular vesicles per square micron of axoplasm of the section through a nerve fiber in the control experiments averaged 123 ± 17. Sodium hydroxybutyrate had no effect on NA reserves, the intensity of fluorescence of the mediator in the nerve fibers, or the number of synaptic vesicles containing the mediator.

After the addition of tyramine the NA content in the vas deferens was reduced by more than 40%. The number of granular synaptic vesicles and the intensity of fluorescence of the mediator in the adrenergic nerves also fell. The reserves of mediator were restored 30 min after addition of NA in concentrations of $3 \cdot 10^{-6}$ and $9 \cdot 10^{-5}$ M, or even exceeded their initial level. The number of vesicles storing mediator increased. The intensity of fluorescence of the nerve fibers was fully restored.

TABLE 1. Effect of Sodium Hydroxybutyrate on Content and Uptake of NA in Isolated Rat vas deferens

Substance and concentration (M)	NA concentration		Intensity of fluorescence (in conventional units)	No. of granular synaptic vesicles per square micron (in % of control)
	in μg/g weight of tissue	in % of control		
Control	9,7±0,7*	100	++++	100±14
Sodium hydroxybutyrate $4.4 \cdot 10^{-3}$	9,5±0,9	98	++++	106±15
Tyramine $2 \cdot 10^{-3}$	5,5±0,6	57	++(+)	18±7
Tyramine + NA $3 \cdot 10^{-6}$	10,9±1,7	112	++++	40±8
Tyramine + NA $9 \cdot 10^{-5}$	13,2±2,3	136	++++	85±11
Tyramine + sodium hydroxybutyrate $4.4 \cdot 10^{-3}$ + NA $3 \cdot 10^{-6}$	6,8±0,5	70	++(+)	27±8
Tyramine + sodium hydroxybutyrate $4.4 \cdot 10^{-3}$ + NA $9 \cdot 10^{-5}$	13,8±1,8	142	++++	72±15

Legend. ++++) Bright fluorescence, +++) moderate, ++) weak, +) very weak.

Note. Incubation with tyramine ($2 \cdot 10^{-4}$ M) in all experiments contined for 2 h; incubation with NA for 30 min; in all experiments sodium hydroxybutyrate was added 15 min before NA.

*Confidence limits of mean for P = 0.05.

Sodium hydroxybutyrate, if added before NA, prevented the uptake and accumulation of exogenous mediator in the tissues, adrenergic fibers, and synaptic vesicles; however, this effect took place only when NA was present in the medium in low concentration ($3 \cdot 10^{-6}$ M). If NA was added in a concentration of $9 \cdot 10^{-5}$ M, sodium hydroxybutyrate did not prevent NA from penetrating into the nerve fibers and synaptic vesicles.

It can be concluded from these results that sodium hydroxybutyrate does not affect the reserves of adrenergic mediator in the tissues, nerve fibers, and synaptic vesicles. However, this compound can block the accumulation of exogenous NA in sympathetic fibers if the mediator is present in the medium in low concentration.

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