

BIOCHEMISTRY AND BIOPHYSICS

THE EFFECT OF SOME LOCAL ANESTHETICS ON PROTEIN METABOLISM IN STRATIFIED SQUAMOUS EPITHELIUM

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Despite many achievements in the field of the pharmacology of local anesthetic drugs, we are still ignorant of the intimate biochemical and physicochemical mechanism of their action. In order to explain the mechanism of local anesthetic action it would be useful to find out how tissue metabolism is affected by local anesthesia. We therefore set out to study the effect of some local anesthetics on protein metabolism in the tissues.

In this work we used two compounds belonging to quite distinct classes of local anesthetic: cocaine and xylocain. The latter is new and is identical with the foreign local anesthetic xylocaine. In its chemical structure it is the hydrochloride of diethylamino-2,6-dimethylacetanilide.* According to our experimental and clinical findings and the reports in the literature, xylocaine is distinguished by its low toxicity and high local anesthetic power [4]. In addition, by contrast with cocaine, in its chemical structure it is an amide. It was essential to discover whether any selective action of the different local anesthetics was observed on the intensity of metabolic processes, or whether the local anesthetics act irrespectively of their chemical and physicochemical properties.

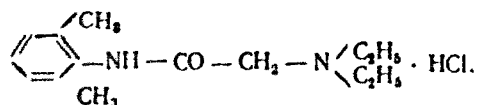
As the subject of the investigation we chose stratified squamous epithelium from the mucous membrane of the tongue of the white rat, which is known to undergo constant and intensive regeneration. This feature makes this tissue very suitable for the study of metabolic relationships.

In solving our problem we employed the method of labelled atoms. An index of the intensity of the metabolic processes going on in the tissues is the quantity of radioisotope taken up by them. In this case we estimated the rate of resynthesis of proteins by the quantity of methionine labelled with radioactive sulfur (S^{35}) taken up by the tissue. Of the methods of working with labelled atoms used in biology, the one which has been most widely used recently is that of autoradiography, which enables not only the intensity of incorporation but also the character of the distribution of the radioisotope in the tissues to be estimated. This makes it possible to relate the metabolic processes occurring in the tissue accurately to its histological structure and thereby to follow the metabolic changes resulting from pharmacological agents.

EXPERIMENTAL METHOD

Experiments were performed on white rats weighing 100-150 g. The animals were starved for 8-10 hours

*Formula of xylocaine:



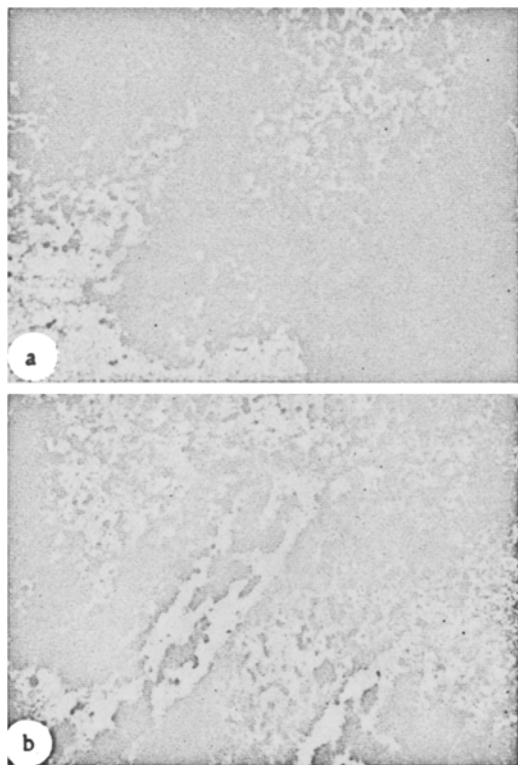


Fig. 1. Tracer autoradiographs of the epithelium of the mucous membrane of the dorsum of the tongue of white rats two hours after injection of methionine labelled with S^{35} : a) normal; b) after local anesthesia. Exposure of photographic emulsion - three days. Objective 60 immersion. Ocular 7.

sion formed by disintegration of the radioactive sulfur) for a trace film but not enough for a contrast film, i.e., they could not be treated by counting the tracks nor by determining the density of blackening.

The intensity of incorporation of the radioisotope in the tissues was determined from the contrast autoradiographs by the density of blackening of the photographic emulsion on the section, which was measured by a microphotometer MF-4 and registered in units of the logarithmic scale of the microphotometer.^{**} In addition to visual observations and registration of the indications on the microphotometer scale, we recorded simultaneously the density of blackening of the test areas of tissue on a photographic film or plate. On the trace autoradiographs the intensity of incorporation of radioactive sulfur was determined by counting under a microscope with a grid of an ocular micrometer the number of tracks appearing in one square measuring $100 \mu^2$.

EXPERIMENTAL RESULTS

The rate of incorporation of radioactive sulfur in stratified squamous epithelium of the tongue of white rats in normal conditions was different in the various strata of the epithelium. The most intensive incorporation was found in the cells of the basal germinal layer, especially the basal layer itself. On passing from the basal

before the start of the experiment. For the experiment the rats were divided into two groups: experimental and control (with five to six rats in each). Before administration of the radioisotope superficial anesthesia of the mucous membrane of the tongue was carried out in the experimental group of animals by painting it with a 10-20% cocaine solution or with xylocaine. As a rule, anesthesia developed rapidly in both cases and was sufficiently prolonged. Absence of pain in response to stimuli in the form of a prick with a needle served to indicate anesthesia. The mucous membrane of the tongue of the control animals was painted with isotonic saline. Next the animals of both groups were given intravenous injections of methionine, labelled with radioactive sulfur, in a dose of $0.5 \mu\text{C}$ per 1 g body weight. Two hours after the injection of methionine the rats were decapitated and pieces of the anterior portion of the tongue fixed in Carnoy's solution. After dehydration the material was embedded in paraffin wax and histological sections of a thickness of 7μ prepared from it. On the sections was applied a liquid photographic emulsion by a method described by N.D. Gracheva, L.N. Zhinkin and E.I. Shcherban'.^{*} In the preparation of the substrate and the emulsion, and the developing and fixing of the material, we followed the instructions of the Research Cine-Photo Institute.

Depending on the duration of exposure of the emulsion, trace or contrast autoradiographs were obtained. Autoradiographs were prepared with exposures of 1, 3, 5, 10 and 25 days. Those with exposures of one and three days were trace films - those with exposures of 10 and 25 days - contrast. Exposure of five days was found to be unsatisfactory: the autoradiographs contained too many tracks (traces in the photographic emul-

^{*}Liquid emulsion type R, issued by the Research Cine-Photo Institute.

^{**}The logarithmic scale of the MF-4 microphotometer is drawn up according to a formula in which the density of blackening is expressed as a logarithmic ratio of the intensity of a beam of light passing through the emulsion outside the preparation to the intensity of a beam of light passing through the area of tissue to be examined.

layer to the surface of the mucous membrane the intensity of incorporation gradually fell and was quite insignificant in the cells of the stratum corneum. The autoradiographic findings on the character of distribution of the radioactive sulfur incorporated in the epithellum of the tongue in rats in normal conditions agree with those reported in the literature [1, 2, 3, and others]. There was a marked change in the intensity of incorporation of radioactive sulfur by the cells of the stratified squamous epithellum of the tongue during local anesthesia. In the experimental animals the incorporation of methionine labelled with radioactive sulfur took place more slowly than in the controls.

The table shows the mean results of the track counts for ten squares of the ocular micrometer grid, i.e., for $1000 \mu^2$, taken from the 100 counting units (squares) and the mean results of photometry obtained from ten (for each exposure) contrast autoradiographs.

Change in the Intensity of Incorporation of Radioactive Sulfur in the Cells of the Stratified Squamous Epithellum of the Mucous Membrane of the Tongue in White Rats as a Result of Local Anesthesia

Epithellal layer*	Average number of tracks per $1000 \mu^2$ (trace autoradiographs)						Average density of blackening in units of the logarithmic scale of the MF-4 microphoto- meter (contrast autoradiographs)					
	1 day			3 days			10 days			25 days		
	control	expt.	expt.**	control	expt.	expt.	control	expt.	expt.	control	expt.	expt.
			control (%)			control (%)			control (%)			control (%)
First layer	16.4	9.0	54.9	32.7	19.0	58.1	34	23	67.6	57	48	71.6
Second layer	17.0	8.3	48.8	29.6	17.2	58.1	24	16	66.6	52	36	69.2

*The first layer corresponds histologically to the basal portion of the stratum germinativum, i.e., to the cells of the basal and prickle-cell layers; the second layer embraces the cells of the prickle-cell layer and the adjacent stratum granulosum.

** $\frac{\text{Experiment}}{\text{control}}$ (%) is the ratio between the results in the experimental and control animals expressed as a percentage.

The difference in the intensity of incorporation of radioactive sulfur which we observed between the control and experimental groups was shown in both photometry and track counts. If we take the intensity of incorporation in the control animals to be 100%, in the experimental animals, according to the results of track counts, it was reduced in various layers to 48.8-58.9%, and, according to photometry, to 66.6-71.6%.

As an illustration of the above, in Figure 1 are shown photographs of trace microautoradiographs of control (a) and experimental (b) animals - even with the naked eye the difference in the intensity of incorporation of radioactive sulfur is obvious. Similar findings were obtained by photographic recording of the density of blackening of contrast microautoradiographs by means of a MF-4 microphotometer (Figure 2). It is clear that the density of blackening in the control (a) reaches 75 divisions of the logarithmic scale of the microphotometer, while in the experiment (b) it hardly reaches 40. Comparing the findings obtained by photometry with those obtained from track counts, we see that they are in complete agreement.

No essential difference between the intensity of incorporation in the experimental groups could be detected: it appeared that xylocaine and cocaine have an equal action on the intensity of the metabolic processes, slowing the rate of resynthesis of proteins to about the same degree.

On the basis of the experiments reported in this paper it can be concluded that our method of autoradiography enables us to judge the character of the changes arising in certain cell strata, and also their intensity. In studying the effect of local anesthesia in the tissue metabolism we have proved that the intensity of the metabolic processes taking place in the epithellum of the tongue is diminished as a result of local anesthesia. However the metabolic relations observed in normal animals are preserved.

It is my pleasant duty to express my profound thanks to Prof. L.N. Zhinkin and to my colleagues in the laboratory of histology, under his direction, at the Institute of Experimental Medicine of the Acad. Med. Sci. USSR

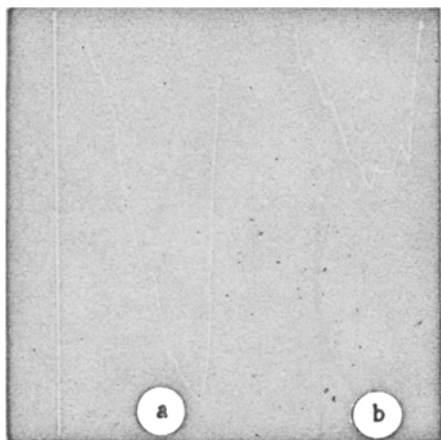


Fig. 2. Photometric curves of contrast autoradiographs obtained by photographic recording on a MF-4 microphotometer two hours after injection of methionine labelled with sulfur (S^{35}): a) normal; b) during local anesthesia. Exposure of the emulsion - 25 days. Along the vertical axis are shown the divisions of the logarithmic scale of the microphotometer (explanation in text).

for frequent advice and help during the course of this work.

SUMMARY

A study was carried out to discover the effect of local anesthesia on the intensity of incorporation of S^{35} -labelled methionine into the cells of stratified epithelium of the tongue mucosa in white rats. It was established that the velocity of incorporation of S^{35} into the cells of stratified epithelium is retarded under the effect of local anesthesia (10-20% xylocaine or cocaine). Consequently, the velocity of protein resynthesis in this tissue is also retarded. However, the character of distribution of S^{35} in various layers of the epithelium is not changed. No pronounced difference was noted in the intensity of incorporation of S^{35} in administration of cocaine or xylocaine. It was demonstrated that both anesthetics affect the intensity of metabolism in the same way, and retard the resynthesis of protein to about the same degree.

LITERATURE CITED

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