Besides the reduction in the resting tension and the duration of contraction, the positive ionotropic action of the ionphore was also accompanied by changes in the shape of the twitch in response to the stimulus. A discontinuity appeared in the phase of development of contraction and, correspondingly, the positive phase of the first derivative of the contraction was split into two components. According to data in the literature, a biphasic contraction can be obtained in sodium-free solution with an increased Ca^{2+} level through the action of caffeine, [1]. The authors cited explain the nature of this contraction by the participation of two different Ca^{2+} fractions in the contractile act: 1) intracellular Ca^{2+} accumulated in the sarcoplasmic reticulum, 2) extracellular Ca^{2+} arriving via the calcium channel in the phase of depolarization of the cell membrane. This hypothesis is confirmed by inhibition of the second phase of contraction by agents blocking the calcium current (Mn^{2+}, La^{3+}, D-600) and the insensitivity of the first phase of contraction to these blocking agents. The discontinuity in the phase of development of contraction observed in the presence of A23187 is probably similar in nature to the biphasic contraction described above. This explanation is confirmed by experiments using D-600, which abolishes the second component of the twitch but has little effect on the first. The biphasic character of development of contraction under the influence of A23187 can probably be explained by a change in the relative contributions of the reticular and extracellular calcium in myocardial contraction.

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EFFECT OF BARBITURATES ON SELECTIVE SECRETION OF RAT SALIVARY GLANDS AFTER ADMINISTRATION OF ANIONS

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Secretion of labeled anions and their metabolites in the saliva of adult Wistar rats was studied. The salivary glands are characterized by high selectivity of secretion of materials. After subcutaneous injection of [^{14}C]acetate, [^{32}P]orthophosphate, [^{35}S]thiocyanate, and [^{131}I]iodide, in control animals under physiological conditions ^{14}C is concentrated in the mixed saliva 2.5-6 times more than in the blood, the ^{32}P level in the saliva is 1/5-1/20 of the blood level, and the ^{131}I and ^{35}S indices occupy an intermediate position between those of ^{14}C and ^{32}P. Penetration of labeled anions and their metabolites into mixed saliva from the blood was considerably altered in rats receiving barbital sodium (medinal): the relative activity of ^{14}C, ^{35}S, and ^{131}I in the saliva compared with the blood was lower in these rats than in the control animals, but the relative activity of ^{32}P in the saliva compared with the blood was higher than in the control rats.

KEY WORDS: secretion of saliva; labeled anions; selectivity; barbiturates.

Investigation of the effect of narcotics on selective secretion of anions by the salivary glands is not only of special interest in connection with the study of salivary gland function, but it is also important in the context of the study of the principles governing neurogenic influences on permeability of membranes and secretory

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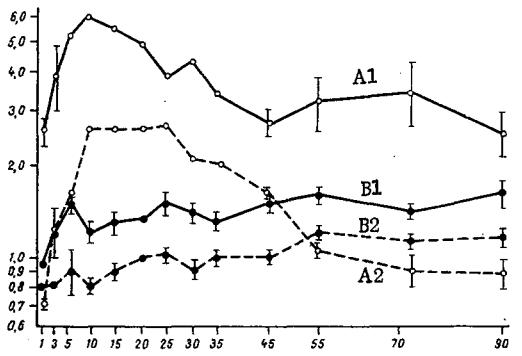

Fig. 1

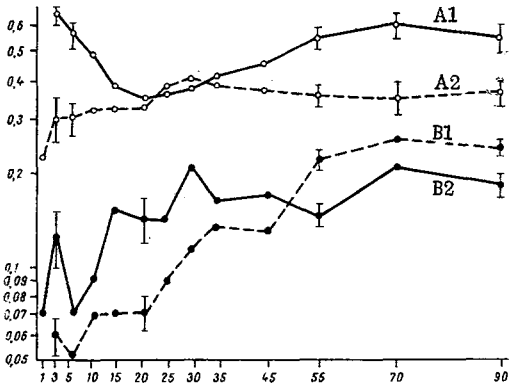

Fig. 2

Fig. 1. Effect of barbital sodium (medinal) on relative activity of saliva/blood in rats after injection of [^{14}C]acetate (A) and Na^{131}I (B). Here and in Fig. 2: 1) without barbiturate, 2) barbital sodium; abscissa, time of taking blood after injection of isotope (in min); ordinate, relative activity saliva/blood.

Fig. 2. Effect of barbital sodium (medinal) on relative activity of saliva/blood of rats after injection of KCN^{35}S (A) and $Na_2H^{32}PO_4$ (B).

structures. However, no such information can be found on anions and the salivary glands.

With these considerations in mind it was decided to compare the selective secretion of the salivary glands after injection of various labeled anions into rats either in the waking state or treated with narcotics. The pattern of selective excretion of various substances by the salivary glands has been studied more thoroughly in man and dogs than in rats and other animals [7]. Rats were chosen because a sufficient quantity of stimulated saliva can be obtained, but also because it is easy to select a large number of individuals of similar breed, age, and other characteristics, so that the scatter of the data is reduced. It is practically impossible to choose the same number of dogs, and very little saliva is produced by guinea pigs, hamsters, and albino mice.

EXPERIMENTAL METHOD

Experiments were carried out on 96 adult Wistar albino rats. Salivation was induced by subcutaneous injection of the muscarinic cholinomimetic pilocarpine, in a dose of 0.05 ml of a 1% solution/100 g body weight. Solutions of labeled compounds were injected 5 min after the injection of pilocarpine, in the following doses (in μCi/100 g body weight): Na^{131}I 0.5, KCN^{35}S 2.5, $Na_2H^{32}PO_4$ 1, and ^{14}C-acetate 20. Portions of saliva were collected for the periods 1-2, 3-4, 5-6, 7-12, 13-17, 18-22, 23-27, 28-32, 33-37, 38-48, 49-60, 61-80, and 81-100 min, and blood was taken from the caudal vein 1, 3, 5, 10, 15, 20, 25, 30, 35, 45, 55, 70, and 90 min after injection of the isotope. From 0.1 to 0.2 ml of saliva or 0.025 ml blood was applied to the target and dried. β-Activity of ^{14}C, ^{32}P, ^{35}S, and ^{131}I of the saliva and blood was determined on a radiometer with T-25-BFL end-window counter. γ-Radiation from ^{131}I in the thyroid gland was determined intravitally on a scintillation counter with FÉU-19M photoelectronic multiplier, and activated KI crystals. The concentration of the isotopes in 1 ml saliva and blood relative to the injected dose per gram body weight and the concentration of ^{131}I in the thyroid gland relative to the dose injected into the whole rat, as well as the relative activity (RA) of saliva to blood, in per cent, were calculated as previously [2, 5-7].

Mixed saliva flowing freely from the mouth of 48 waking rats was obtained while the animals were fixed under physiological conditions in a special frame [6]. To study the effect of barbiturates, the freely flowing mixed saliva was collected [9] from 48 rats immobilized by intraperitoneal injection of barbital sodium (medinal) in a dose of 20 mg/100 g body weight. The numerical results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

High selectivity of secretion of the various substances by the salivary glands will be clear from the differences of tens or hundreds of times between the values of RA for saliva/blood in the waking rats (Figs. 1 and 2). For instance, penetration of ^{14}C from blood into saliva was an order of magnitude greater than that of ^{35}S and two orders of magnitude greater than that of ^{32}P. The intensity of penetration of ^{131}I from blood into saliva was intermediate between that of ^{14}C and ^{35}S.

TABLE 1. Effect of Barbital Sodium (medinal) on Rate of Secretion of Saliva by Rats, μl min (M ± m)

Rats	Time of collecting saliva (minutes after beginning of experiment)												
	1—2	3—4	5—6	7—12	13—17	18—22	23—27	28—32	33—37	38—48	49—60	61—80	81—100
Waking	79,9	67,8	70,3	40,3	42,4	31,5	31,9	28,9	27,0	22,7	19,5	12,0	6,7
	5,5	4,7	5,9	4,6	3,7	2,8	2,7	2,7	2,6	1,8	2,1	1,8	1,4
Treated with	88,3	86,5	88,7	55,0	58,8	51,3	49,2	41,2	40,6	29,6	29,5	23,5	18,3
medinal	7,3	6,8	9,6	5,3	6,9	4,9	5,0	4,3	4,1	3,3	4,4	4,0	2,9
P	>0,1	<0,05	>0,05	<0,05	<0,05	<0,01	<0,02	<0,05	<0,02	>0,05	<0,05	<0,05	<0,01

The pattern described above is also evidence of species-specific differences in the secretion of anions by the salivary glands of rats, for the concentration of iodide and thiocyanate in the saliva of dogs and man is about 10 times higher than in the blood [7]. In dogs, just as in rats, orthophosphate penetrates poorly into the saliva, whereas in man it does so in large quantities.

Of course, if the isotope is found in the saliva, this does not mean that all of it must have penetrated therein as the injected compound. Metabolism may lead to some degree of transfer of the label into other compounds.

The poor penetration of orthophosphate from blood into saliva can evidently be largely explained by the fact that it is a precursor of high-energy phosphates, nucleic acids, phosphoproteins, phospholipids, and other phosphorus-containing compounds, intensively synthesized in the salivary glands and other organs, and also in blood cells [3, 8, 10, 14, 15]. The high concentration of ^{14}C in the saliva after injection of [^{14}C]acetate evidently also was to some extent attributable to rapid conversion of the acetate into the end products $^{14}CO_2$ and H_2O. Much of the $^{14}CO_2$ is rapidly converted into the anions $H^{14}CO_3^-$ and $^{14}CO_3^{2-}$, the concentration of which in the saliva is many times greater than in the blood [5].

In rats receiving medinal, RA for saliva/blood for all isotopes differed from that in the waking animals (Figs. 1 and 2). For instance, RA of ^{14}C and RA of ^{131}I were lower throughout the experiment in rats receiving medinal. In the experiments with thiocyanate, during the first 10 min and at the second hour after administration of the barbiturate, less ^{35}S had penetrated into the saliva than in the waking rats. Not until after 20-25 min did this difference disappear. The reaction of phosphate was biphasic: during the first half of the experiment RA of saliva/blood was higher in the waking rats, but in the second half, on the other hand, it was higher after administration of barbiturate.

The mechanism of the modifying effect of barbiturate can evidently be explained not only by the direct effect of medinal on the permeability of the acinar cells and cells of the salivary ducts, but also by the action of metabolic changes in the salivary glands and other organs on RA of saliva/blood. Changes in the rate of secretion of saliva also were very important.

In the case of ^{131}I, this is clearly seen in the metabolic changes in the thyroid gland, as a result of which the blood iodine level should have risen, for the uptake of iodine into the thyroid gland was reduced by 50-75% after administration of barbiturate. For instance, the accumulation of ^{131}I in the thyroid gland (as a percentage of the dose administered to the rat) in waking rats and in animals receiving barbital sodium was 22.9 ± 2.5 and 10.1 ± 0.5%, respectively, after 1 h, 29.2 ± 2.3 and 11.8 ± 1.0% after 2 h, 38.0 ± 2.5 and 11.8 ± 1.3% after 4 h, and 42.6 ± 2.8 and 11.8 ± 1.6% after 6 h.

In rats the concentration of ^{131}I in the saliva fell after injection of medinal also because of an increase in the rate of secretion of saliva (Table 1). This inverse relationship between the intensity of secretion of ^{131}I and the rate of secretion of saliva [11, 12] is a special case of the general rule of direct or reciprocal relations between the quantity of the secreted component and the rate of secretion of saliva.

Sometimes the method of pilocarpine stimulation of saliva secretion is used to study the composition and rate of secretion of saliva in rats only after administration of barbiturates, without a physiological control [9, 13], although the authors of the method point out, and the present writers confirm, that some animals die under these conditions. Moreover, the present investigation showed that the parameters of selective salivary secretion induced by pilocarpine, a drug which is known to act selectively on muscarinic cholinergic receptors [1], are not identical in waking rats and in animals whose synaptic transmission of excitation has been modified by sedatives [4]. Consequently, results obtained in animals immobilized by barbiturates cannot be used without verification in waking rats to judge precisely the natural physiological level of secretion of ions and compounds in the saliva.

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