THE SEARCH FOR DRUGS TO DEPRESS THE ACTION OF ANTIDIURETIC HORMONE

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According to A. G. Ginetsinskii's hypothesis, an increase in the permeability of the wall of the renal tubules as a result of the action of antidiuretic hormone (ADH) is brought about by the secretion of hyaluronidase and subsequent depolymerization of intercellular hyaluronic complexes [2, 11]. The discovery of this mechanism of action of ADH made possible the search for ADH inhibitors among various antihyaluronidase preparations. Recently A. G. Ginetsinskii and V. F. Vasil'eva showed that ascorbic acid and heparin—both hyaluronidase inhibitors—possess a diuretic action [4].

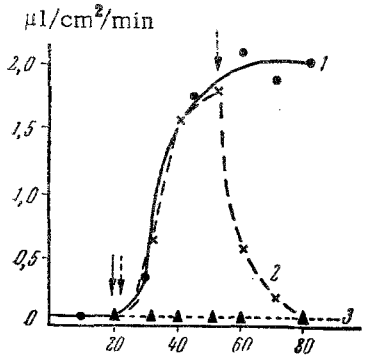

Fig. 1. Depression of the effect of pituitrin by diethazine. Along the axis of abscissas) time (in minutes); along the axis of ordinates) volume of water (in µl) passing through 1 cm^2 of bladder surface per min. 1) Change in permeability after addition of 1 milliunit pituitrin; 2) the same diethazine ($1 \cdot 10^{-3}$ M) added to the solution at the 30th min; 3) 1 milliunit pituitrin and $1 \cdot 10^{-3}$ M diethazine added simultaneously. The arrows denote the moment of addition of the test drugs to the solution bathing the serous surface of the bladder.

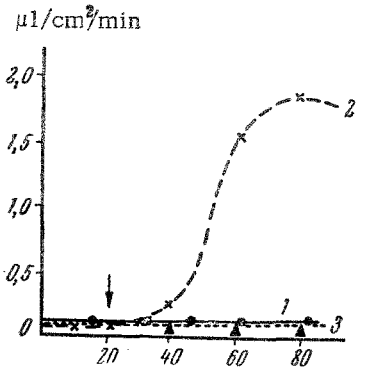

Fig. 2. Effect of histamine on permeability of the urinary bladder. Along the axis) of abscissas) time (in minutes); along the axis of ordinates) volume of water (in µl) passing through 1 cm^2 of bladder surface per min. 1) Histamine in a concentration of $1 \cdot 10^{-3}$; 2) pituitrin (1 milliunit)+ histamine ($1 \cdot 10^{-3}$ M); 3) pituitrin (1 milliunit+ histamine ($1 \cdot 10^{-3}$ M)+ diprazin ($1 \cdot 10^{-3}$ M).

Pituitrin increases the permeability of the urinary bladder of amphibians to water because of an increase in intercellular permeability [9]. Functionally, this object may be regarded as the analogue of the terminal divisions of the renal tubules in mammals, and it is therefore suitable for the study of the mechanism of action of ADH. The effect of ADH on the permeability of the wall of the urinary bladder is depressed by various antihyaluronidase prep-

arations, the strongest ADH inhibitors being the antihistamine drugs: SPOFA, mephlophenhydramine, etc. [9] which possess the power of inhibiting the spread of dyes in the skin of the guinea pig following intradermal injection of hyaluronidase [14].

In the present study, the urinary bladder of the frog Rana temporaria was used as test object for the search for compounds depressing the action of ADH, and for the analysis of the relationship of their chemical structure to their antipituitrin activity.

TABLE 1. Antipituitrin Effect of Certain Drugs in a Concentration of $1 \cdot 10^{-3}$ M (as Change in Weight of the Frog's Bladder in Percent During Action of Pituitrin for 1 h)

Substance	M ± σ	P
Pituitrin (1 milliunit/ml)	63.1 ± 2.7	
Diprazin (Phenergan)	0.5 ± 0.2	< 0.001
Multezin	63.6 ± 15.2	> 0.5
Chlorpromazine	0.2 ± 0.05	< 0.001
Chloracizine	8.1 ± 5.2	< 0.001
Diethazine	3.3 ± 0.5	< 0.001
Diphasin	10.3 ± 3.9	< 0.001
Mefazin	64.1 ± 6.1	> 0.5
Cyclizine	5.2 ± 3.1	< 0.001
M-(N'-diethylaminoethyl-alanyl)-phenothiazine	11.2 ± 4.9	< 0.002
N-(dimethylaminoacetyl)-m-chloroaniline	40.3 ± 6.7	< 0.02
10-Diethylaminoethylacridine	57.4 ± 7.5	> 0.5
Benadryl	8.9 ± 4.1	< 0.001
Antihistamine	17.1 ± 2.2	< 0.001
Mephenhydramine	9.9 ± 2.1	< 0.001
Mephlophenhydramine	5.3 ± 2.2	< 0.001
Antistin	14.7 ± 2.4	< 0.001
Diazoline (Omeril)	26.1 ± 7.2	< 0.01
Pentafen (Merpanit)	10.4 ± 5.3	< 0.001
Merpanit	61.1 ± 10.9	> 0.5
Aprenal	16.6 ± 5.1	< 0.005
Mesphenal	55.7 ± 10.1	> 0.5
Tifen	8.5 ± 2.8	< 0.001
Tifen methylsulfomethylate	52.7 ± 10.7	> 0.5
Benzacine	48.9 ± 16.4	< 0.1
Antrenyl	54.2 ± 10.1	> 0.5
Diazil	37.1 ± 13.8	< 0.05
Diazil methylsulfomethylate	71.2 ± 3.9	> 0.5
Dimethylamino-2,3-dimethylpropylbenzylate	21.4 ± 9.2	< 0.02

EXPERIMENTAL METHOD

The frog's bladder was filled through a tube inserted into the cloaca with Ringer's solution diluted 1:10 with distilled water. Both halves of the bladder were ligated separately and transferred to vessels containing constantly aerated Ringer's solution. The measure of the permeability of the bladder wall was the volume of water in microliters absorbed along the osmotic gradient through 1 cm^2 of bladder surface per minute, or the decrease in the weight of the bladder in percent during a standard experimental period. Pituitrin P (1 milliunit/ml) and all the substances for testing were always added to the solution bathing the serous surface of the bladder.

EXPERIMENTAL RESULTS

As a result of the osmotic gradient between the contents of the urinary bladder and the Ringer's solution bathing its serous surface, movement of water took place from the bladder, at an average rate of 0.05 $\mu l/cm^2/min$. The

addition of pituitrin increased the permeability of the bladder wall for water tenfold (Fig. 1). This effect of ADH was totally suppressed by certain antihistamine drugs (see Fig. 1 and Table 1).

As a first step it was essential to know whether the antipituitrin effect of these substances was connected with their antihistamine action. However, histamine itself, even in a concentration of $1 \cdot 10^{-3}$ M, did not increase the permeability of the bladder wall (Fig. 2); a mixture of $1 \cdot 10^{-3}$ M diprazin (promethazine hydrochloride) and $1 \cdot 10^{-3}$ M histamine had the same antipituitrin effect as diprazin alone. Furthermore, during the first 10-20 min, histamine slightly depressed the action of pituitrin (see Fig. 2). Hence, the antipituitrin effect of the antihistamine drugs apparently was not connected with their antihistamine action.

TABLE 2. Antipituitrin Action of Substances (Depression of Effect of 1 milliunit of Pituitrin, in Percent) and Their Antihistamine Properties

Substance	Concentration (M)				Comparative strength of antihistamine properties*
	$1 \cdot 10^{-3}$	$1 \cdot 10^{-4}$	$1 \cdot 10^{-5}$	$1 \cdot 10^{-6}$	
Diprazin (Phenergan)	100	75	32	0	100
Chlorpromazine	100	59	0	–	1.5
Diethazine	94	85	0	–	1
Chloracizine	87	58	0	–	0.6
Diphasin	84	38	0	–	0.3
Benadryl	86	22	0	–	50
Antihistamine	73	–	–	–	66
Mephlophenhydramine	92	–	–	–	
Mephenhydramine	84	0	–	–	133
Diazoline (Omeril)	60	0	–	–	
Cyclizine	90	28	0	–	
Aprenal	74	38	0	–	
Pentafen (Merpanit)	84	51	–	–	
Antistin	70	20	–	–	27
Tifen	97	85	46	11	

* The figures show the relative ability to diminish the spasm of the isolated intestine of a guinea pig, caused by histamine, by 50%. The activity of diprazin is taken as 100 [1, 10].

In the next series of experiments substances closely similar to the antihistamine compounds already investigated were chosen, but possessing at the same time a weak antihistamine action or none whatever (see Table 1). It was found that diphasin, chlorpromazine, and other phenothiazine derivatives, which are not typical antihistamine compounds, also possessed a marked antipituitrin effect. On the other hand, the antihistamine drug diazolin had a weak antipituitrin action, whereas substances practically without antihistamine activity (aprenal, pentafen, tifen, etc.) had a strong antipituitrin action.

Comparison of the relative strength of the antipituitrin action of the tested compounds (Table 2) showed that tifen and diprazin had the strongest action (Fig. 3). An absolute lack of correlation was found between the strength of the antipituitrin effect and the intensity of the antihistamine properties of the substances (Table 2). Consequently, the antihistamine action was not concerned in the mechanism of the antipituitrin effect.

Among the compounds tested there were seven pairs, each of which included a tertiary amine and the corresponding quaternary ammonium base, which differed from the tertiary amine only by the fact that it contained an additional radical on its nitrogen atom (a methyl group), and consequently possessed a free positive charge in this position. The quaternary ammonium bases (Multezin—the same as diprazin—mefazin, Merpanit, mesphenal, tifen methylsulfomethylate, Antrenyl, and diazil methylsulfomethylate) in a concentration of $1 \cdot 10^{-3}$ M had no effect on the increased permeability of the urinary bladder of the frogs caused by pituitrin to water, whereas their

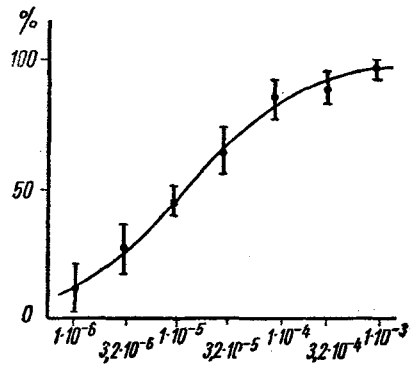

Fig. 3. Relationship between the antipituitrin effect of tifen and its concentration. Along the axis of abscissas) concentration of tifen (logarithmic scale); along the axis of ordinates) depression of pituitrin effect (in percent).

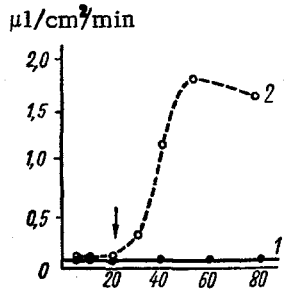

Fig. 4. Absence of antipituitrin effect of quaternary ammonium derivatives. Along the axis of abscissas) time (in minutes); along the axis of ordinates) volume of water (in µl) passing through 1 cm^2 of the bladder wall per min. 1) Depression of effect of 1 milliunit of pituitrin by addition of $1 \cdot 10^{-3}$ M diphasin; 2) pituitrin (1 milliunit)+ mefazin (quaternary ammonium derivative of diphasin) in a concentration of $1 \cdot 10^{-3}$ M.

tertiary analogues (diprazin, diphasin, pentafen, aprenal, tifen, benzacine, diazil) possessed high activity (Fig. 4, see Table 1), although as regards their antihistamine action they were weaker than their quaternary analogues [8].

Pituitrin, which acts on the cells of the urinary bladder, increases the intercellular permeability for the movement of osmotically free water, probably by causing the secretion of an exo-enzyme similar to hyaluronidase [9]. The antihistamine SPOFA, which has an antihyaluronidase action in vivo [14] and gives an antipituitrin effect on the urinary bladder [9], does not depress hyaluronidase in experiments in vitro using a viscosimetric method. Consequently, the action of these drugs evidently was not associated with the direct depression of hyaluronidase.

The drugs tested depressed the effect of pituitrin, not only when given at the same time as the hormone, but also when administered a short time after the beginning of the action of the pituitrin (see Fig. 1). They probably halted the secretion of the exo-enzyme without, at the same time, preventing regeneration of the intercellular substance.

We know that the increase in diuresis brought about by existing diuretics is based on the depression of sodium reabsorption and increased excretion of sodium in the urine (mercurial diuretics, acetazolamide, hypothiazide, etc.) [3]; under these circumstances water passively follows the osmotically active substances. The excretion of osomotically free water in these conditions is usually prevented by the secretion of ADH, causing an increase in reabsorption of water [6]. However, no drugs are yet available for clinical use which can depress the peripheral effect of ADH and thereby suppress the reabsorption of osmotically free water.

Our results demonstrate that among the various groups of pharmacologically active substances there are compounds which, in concentrations of $1 \cdot 10^{-5}$ to $1 \cdot 10^{-6}$ M, have a marked antipituitrin action and which cause considerable depression of the transport of osmotically free water through the wall of the frog's bladder.

The antipituitrin action of one of the most active substances in our experiments (diprazin) has also been demonstrated in experiments on dogs [5]: a unilateral diuretic reaction was found after injection of diprazin into the renal artery.

Hence, there are grounds for considering that these substances may give a clue to assist the search for "water" diuretics—drugs which, in conjuction with existing diuretics depressing the reabsorption of salts, will prove particularly useful for overcoming the oliguria caused by an increased secretion of ADH.

Analysis of the relationship between the chemical structure and antipituitrin action of these drugs shows that the most obvious conclusion is the inefficacy of the quaternary ammonium bases. These bases pass with difficulty through biological barriers [7, 13]. This suggests that in our experiments the quaternary ammonium salts were ineffective because of their inability to penetrate inside the cells. In all probability the antipituitrin action is due to depression of one of the links in the chain of secretion of an hyaluronidase-like enzyme in response to the action of ADH on the cell.

The results indicate that substances having an antipituitrin action are weakly ionized by lipoidophilic compounds. Bearing in mind the relatively high efficacy of all the phenothiazine derivatives that were studied, and also their metabolic effects [12, 15], it may be supposed that a search for antipituitrin substances among this group

will be very profitable. However, such a search will be still more profitable if it proves possible to discover the mechanism of their action and to identify the biological systems with which the manifestations of the pituitrin effect and the action of inhibitors are associated.

SUMMARY

In creating the osmotic gradient between the mucosa and the serous surface of the urinary bladder of the frog the flow of water from the bladder may be intensified by adding pituitrin into the solution, irrigating the serous surface. Addition of some changes to the solution considerably decreases this effect of pituitrin. The most marked antipituitrin action (of the 30 agents studied) is possessed by diprazin (phenergan) and tifen, which in a concentration of 1.10^{-5} to 1.10^{-6} decreased the effect of pituitrin. The antipituitrin effect is not connected with the intensity of the antihistaminic properties of the substances studied. In passing from the tertiary amines to the corresponding quaternary ammonium bases the antipituitrin action disappears. The data obtained may serve as a basis for the search for diuretics capable of depressing the antidiuretic hormone and of increasing the excretion of osmotically free water.

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