The Antidipsic Effect of Sulphonamides Implanted in the Hypothalamus of Rats

In previous publications from this laboratory, the effect of a variety of drugs on the water intake of rats was studied. The compounds were implanted bilaterally into the hypothalamus, and spontaneous water consumption, as well as the response to hypertonic saline, was measured. The most effective antidipsic agents were the cardiac glycosides, ouabain[1] and digitoxin[2]. Ethacrynic acid had a somewhat smaller influence on drinking[2], while furosemide[2] and hydrochlorothiazide were much less active[3]. It was established that the antidipsic effect of drugs implanted into the hypothalamus does not follow the order of their diuretic activity, e.g. furosemide is a much stronger diuretic than hydrochlorothiazide[4], but both suppress drinking with about the same efficiency. Since the 2 latter drugs are both sulphonamido derivatives, it appeared of interest to study other members of this group.

As shown in Table I, the compounds tested include sulphonamides with different pharmacological activities, such as diuretics (chlorothiazide and its dihydro derivative), inhibitors of carbonic anhydrase (diamox), hypoglycemic agents (tolbutamide and chlorpropamide) and bacteriostatics (sulphadiazine and homosulphanilamide). For the first 3 or 4 days after implantation of the drug into the hypothalamus, the animals were allowed only water ad libitum; thereafter food was also supplied.

Control animals received a bilateral deposit of talc. From the second to the fourth day, while fasting, the controls drank between 30–40 ml water/kg/24 h. Thereafter, when food was again provided, the daily water intake rose to about 110 ml/kg. In the light of these findings, only a reduction of the daily consumption to less than 20 ml/kg during the fasting period, was considered significant.

The data of Table I establish the following order of antidipsic activity: chlorpropamide > homosulphanilamide ≈ diamox > sulphadiazine ≈ hydrochlorothiazide > tolbutamide > chlorothiazide. However, even bilateral application of 200 µg of the most effective drug, chlorpropamide, suppressed drinking for about 5 days only, and the rats recovered completely. This result resembles our previous observations on furosemide[2]. At the height of the antidipsic action, the response to hypertonic saline (50 ml/kg of 3% NaCl s.c.) was also strongly reduced (Table II).

It is evident from the present results that the suppression of thirst is unrelated to any one of the known pharmacological effects of the compounds examined. In this connection it is significant that the substituent attached to the *p*-position of the benzene ring does not play any special role. Likewise, the group attached to the amide nitrogen does not appear to exert any decisive influence on the antidipsic activity. The only common feature is the aromatic sulphonamide group. The importance of this substituent is further supported by the observation that diazoxide, a chlorothiazide without a sulphonamido substituent, is devoid of any antidipsic activity[2]. It is thus suggested that all the compounds tested have a common mechanism which involves inhibition of an unknown biochemical reaction by the group $-SO_2NH-$.

[1] F. Bergmann, M. Chaimovitz, A. Costin, Y. Gutman and Y. Ginath, Am. J. Physiol. *213*, 328 (1967).

[2] F. Bergmann, A. Zerachia, Y. Gutman and M. Chaimovitz, J. Pharmac. exp. Ther. *159*, 222 (1968).

[3] Y. Gutman and M. Chaimovitz, Nature *209*, 410 (1966).

[4] J. B. Hook and H. E. Williamson, J. Pharmac. exp. Ther. *148*, 88 (1965).

Table I. Spontaneous water intake of rats after bilateral implantation of sulphonamides into the hypothalamus

Drug	Dose (µg)	Water intake (ml/kg body weight)							
		No. of days							
		1	2	3	4	5	6	7	8
Control	–	20	40	37	35	113[a]			
Tolbutamide	100	26	35	35	19	80[a]			
	200	1	9.2	24	170[a]	135			
Chlorpropamide	100	2.7	5.1	21	98[a]	88			
	200	1.6	0.8	1.6	0.8	1.6[a]	61	43	109
Chlorothiazide	100	12	25	41	123[a]	107			
	200	21	37	47	114[a]	96			
Hydrochlorothiazide	100	7.7	26	52	120[a]	120			
	200	8.2	4.1	5.6	77[a]	52			
Diamox	100	8.5	28	25	31	109[a]	98	105	
	200	0	2.3	7	2.3	23[a]	50	88	
Sulphadiazine	100	6.3	28	34.5	40	106[a]	93	94	
	200	1.5	2.6	10	74[a]	79	100		
Homosulphanilamide	100	0	6.5	45	127[a]	114			
	200	5.2	4.5	11.6	80[a]	66	107		

Groups of 8 rats were used for each test. Under ether anaesthesia, the drug was deposited into the hypothalamus, as described previously[1]. The animals were then supplied with water only for 3–4 days and the daily consumption was measured. The controls received a deposit of 200 µg talc. [a] These figures were obtained after the food supply was reinstalled.

While this study was in progress, ARDUINI et al.[5] reported that peroral chlorpropamide exerts an antidiuretic action on patients with idiopathic diabetes insipidus. The authors discussed the following hypothetical explanations: (a) the drug stimulates formation or release of antidiuretic hormone; (b) it restores the sensitivity of osmoreceptive centres or (c) it mimics the action of ADH in the kidney. However, suppression of thirst as the primary effect of chlorpropamide was not considered. In the light of the results reported in this study, it appears possible that chlorpropamide is better suited for an action on the hypothalamic thirst mechanism than other sulphonamides, such as the chlorothiazides, used previously for the treatment of diabetes insipidus. On the other hand, it should be noted that upon hypothalamic application, even this most active sulphonamide is about 100 times weaker than the cardiac glycosides ouabain and digitoxin[1,2].

Since physiological secretion of ADH is coupled with increased thirst, the question arises whether the antidipsic action of drugs, implanted in the hypothalamus, is accompanied by a suppression of ADH secretion. Experiments on the metabolism of water and electrolytes after hypothalamic application of such drugs will be reported separately.

Table II. Response to hypertonic saline after bilateral implantation of sulphonamides into the hypothalamus

	Water consumption (ml/kg)			
	Talc	Diamox	Tol-butamide	Chlor-propamide
First hour	39	1.4	8.5	0
Second hour	2.4	5	0	0
Third hour	0	1.4	0	0
Total	41.4	7.8	8.5	0

Groups of 4 rats were used for every experiment. Each animal received a deposit of 200 μg on each side of the hypothalamus, while controls received an implant of talc; 48 h later, 50 ml/kg of 3% NaCl were injected s.c. Thereafter, water intake was measured at hourly intervals for 3 h.

Résumé. Divers sulfonamides, implantés dans l'hypothalamus des rats, diminuent la consommation spontanée d'eau ainsi que la réponse à une injection hypertonique. L'activité antidipsique des sulfonamides n'a aucun rapport avec leurs autres propriétés pharmacologiques.

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[5] F. ARDUINI, F. P. J. FERRAZ and J. RODRIGUES, J. clin. Endocr. Metab. *26*, 1325 (1966).

[6] This research forms part of a Ph.D. thesis, submitted by A. ZERACHIA to the Faculty of Medicine of the Hebrew University of Jerusalem.

Neue lokalanästhetisch wirksame basische Cycloalkylester von substituierten Carbanilsäuren

In vorangehenden Mitteilungen[1,2] haben wir Verbindungen vom Typ basischer Carbanilate beschrieben, bei denen der Einfluss von Halogen- und Methylsubstitution am aromatischen Kern untersucht wurde. Um die Erkenntnisse der Beziehungen zwischen Struktur und pharmakologischen Eigenschaften dieser Stoffgruppe zu vertiefen, haben wir eine neue Serie von Verbindungen hergestellt, bei denen die Carbamatgruppe dieser Ester zusätzlich zur Substitution in den Stellen 2, evtl. 2 und 6 des aromatischen Ringes teilweise durch Vergrösserung des alkoholischen Teils des Moleküls sterisch geschützt ist. In allen Fällen wurde diese sterische Abschirmung der Carbamatgruppe im alkoholischen Teil durch basische Cycloalkanole verwirklicht (Tabelle).

Die Verbindungen wurden durch Umsetzung von 0,11 Mol des betreffenden substituierten Phenylisocyanates mit 0,1 Mol des basischen Cycloalkanols gewonnen und die Endprodukte als kristalline Hydrochloride isoliert.

Die lokalanästhetische Aktivität (Oberflächenanästhesie, Kaninchenhornhaut, Standard-Cocain) wurde nach bekanntem Verfahren[3] durch Ermitteln und Vergleichen gleich wirksamer molarer Konzentrationen des Standards (Cocain m/100) bestimmt. Die Toxizität wurde durch s.c. Applikation an weissen Mäusen nach der Methode von LITCHFIELD und WILCOXON[4] ermittelt und als LD 50 ausgedrückt.

Aus den vorläufigen pharmakologischen Prüfungen (siehe Tabelle) ist ersichtlich, dass alle hergestellten Verbindungen wirksamer sind als das Standard-Cocain. Im Vergleich zu monochlor- und dichlorsubstituierten wie auch monomethyl- und dimethylsubstituierten Carbanilaten haben sich die basischen Ester der 2-Chlor-6-methylcarbanilsäure (K 1301, K 1302 und K 1303) als besonders wirksam erwiesen, von denen der Piperidinocyclohexylester (K 1303) als lokalanästhetisch wirksamster Stoff die Aktivität des Standards bei Oberflächenanästhesie 35mal übersteigt. Von den übrigen Verbindungen zeigen noch Piperidinocyclohexylester der 2-Chlorcarbanilsäure (K 1103) und der 2, 4, 6-Trimethylcarbanilsäure (K 1603)

[1] A. BOROVANSKÝ, L. BENEŠ und L. KOPÁČOVÁ, Acta Fac. pharm. bohemoslov. *12*, 179 (1966).

[2] L. BENEŠ, A. BOROVANSKÝ und L. KOPÁČOVÁ, Ceskoslov. farm. *16*, 316 (1967).

[3] C. VRBA und A. SEKERA, Archs int. Pharmacodyn. Thér. *118*, 155 (1959).

[4] J. LITCHFIELD und F. WILCOXON, J. Pharmac. exp. Ther. *95*, 99 (1949).