Physiology and Behavior, Vol. 1, pp. 193-197. Pergamon Press Ltd., 1966 Printed in Great Britain

Effect of Autonomic Blocking Agents and Structurally Related Substances on the "Salt Arousal of Drinking"[1]

D. DE WIED

Department of Pharmacology, Medical Faculty, University of Utrecht, Vondellaan 6, Utrecht, the Netherlands

(Received 10 January 1966)

DE WIED, D. *Effect of autonomic blocking agents and structurally related substances on the "salt arousal of drinking"*. PHYSIOL. BEHAV. 1 (3) 193-197, 1966. -The effect of autonomic blocking agents and structurally related substances was studied in rats in which thirst was produced by the administration of a hypertonic sodium chloride solution. Scopolamine, methamphetamine, amphetamine, chlorpromazine, atropine, mecamylamine, hexamethonium, nethalide, inderal, cocaine, disipal, artane and neobenodine, marshalled in the order of potency blocked or reduced the "salt arousal of drinking". Methylatropine nitrate, dibenzyline, cyproheptadine, deseril and pitressin tannate in oil failed to affect the increased water intake of thirsty rats.

Autonomic blocking agents Amphetamines Salt arousal of drinking Thirst Procaine hydrochloride

NEUROCHEMICAL stimulation of lateral hypothalamic areas by cholinergic agents causes vigorous drinking in the water satiated rat, indicating that cholinergic pathways participate in the regulation of water intake [6, 7, 10]. Additional evidence for this mechanism has been obtained by the demonstration that cholinergic blocking agents inhibit the neurochemical cholinergic stimulation of drinking [7]. In addition, atropine and scopolamine have been shown to reduce water consumption in the rat [1, 14, 15].

Since data on the influence of drugs on water intake are relatively scarce, it was felt of interest to study the effect of autonomic blocking agents and structurally related substances on the water consumption of water satiated rats in which drinking was produced, independent of water deprivation and water loss.

The administration of a hypertonic salt solution has been extensively employed as a method to increase the extracellular osmotic pressure to produce drinking in the rat. Adolph has referred to thirst under these conditions as the "salt arousal of drinking".

The present report deals with the effect of autonomic blocking agents and related substances on the "salt arousal of drinking" in the water satiated rat.

METHODS

Male white rats weighing from 180-220 g were used. Except for the first experiment in which the rat was studied in a lever press situation, animals were placed in individual cages in which they had free access to water from a graduated pipet of 25 ml adapted for this purpose. The environmental temperature was maintained on 25 ± 1 C.

The technique used to induce thirst was the one described by Wayner *et al.* [17]. This consisted of the subcutaneous injection of 3 mEq NaCl administered in a 15 per cent solution which contained 24 mg procaine hydrochloride per ml to eliminate pain associated with the salt injection. As a control solution 0.9% NaCl containing 2.4% procaine was used.

Rats were accustomed to the experimental procedures of handling, injections and placement in the individual cages.

Animals were used 4 times, generally twice a week. The hypertonic salt solution was administered only once a week while the drugs were never given more than once during the whole experimental period in order to avoid possible tolerance.

The drugs were generally injected intraperitoneally immediately after the administration of the hypertonic salt solution. Water intake was measured during the first 2 hr after treatment and recorded every hr to the nearest 0.1 ml.

Following drugs were used: atropine sulphate; methylatropine nitrate; scopolamine hydrobromide; mecamylamine hydrochloride (Merck, Sharp and Dohme); hexamethonium chloride; cocaine hydrochloride; chlorpromazine hydrochloride (largactil, Specia); pitressin tannate in oil (Parke and Davis); phenoxybenzamine (dibenzyline, Smith, Kline and French); dextroamphetamine sulphate; methamphetamine hydrochloride; neobenodine (Brocapharm); methy-

[1]The generous supply of mecamylamine and cyproheptadine (Merck, Sharp and Dohme), of chlorpromazine hydrochloride (Specia), of deseril (Sandoz), of disipal (Brocapharm), of artane (Lederle) and of nethalide and inderal (I.C.I.) is gratefully acknowledged.

sergidum (deseril, Sandoz); cyproheptadine hydrochloride (Merck, Sharp and Dohme); orphenadrine hydrochloride (disipal, Brocapharm); trihexyphenidyl hydrochloride (artane, Lederle); nethalide (alderlin, I.C.I.); propanolol (inderal, I.C.I.).

RESULTS

In the first experiment in which the animals (two groups of six animals each) had to press a lever to obtain water, the effect of the salt arousal of drinking under the present experimental conditions was studied. As can be seen from Fig. 1,

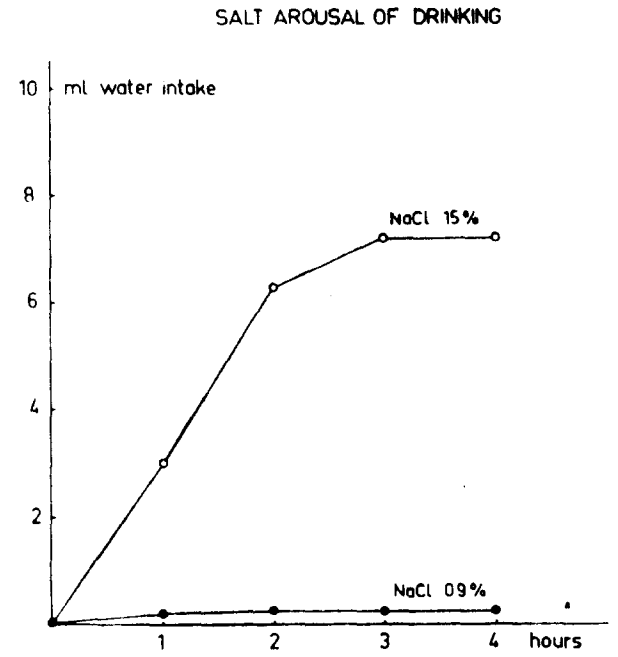

FIG. 1. Effect of 1.2 ml NaCl 15%–2.4% procaine HCl or 1.2 ml NaCl 0.9%–2.4% procaine HCl on water intake of rats in a press lever situation. Six animals in each group.

mean water intake rose sharply during the first 2 hr. Animals generally started to drink 45 min after the administration of the hypertonic salt solution and had finished most of their drinking within 2 hr. Accordingly, it was decided to restrict measurement of water intake to two hr in subsequent experiments.

Subsequent experiments were performed in individual cages in which rats had free access to water from a graduated pipet. Figure 2 shows the effect of graded amounts of sodium chloride on mean water consumption of a group of nine animals. A linear relationship between mEq of injected sodium chloride and water intake was found.

Assuming that cholinergic pathways participate in the regulation of water intake, the effect of atropine and of the peripherally acting derivative methylatropine was investigated. As seen from Fig. 3, atropine sulphate in a dose of 1.25 mg almost completely blocked water consumption in the thirsty animals while the methyl derivative was slightly active.

The influence of similar and other autonomic blocking agents and a variety of structurally related drugs was measured in subsequent experiments. Mean water intakes and their standard errors, are summarized in Tables 1–5.

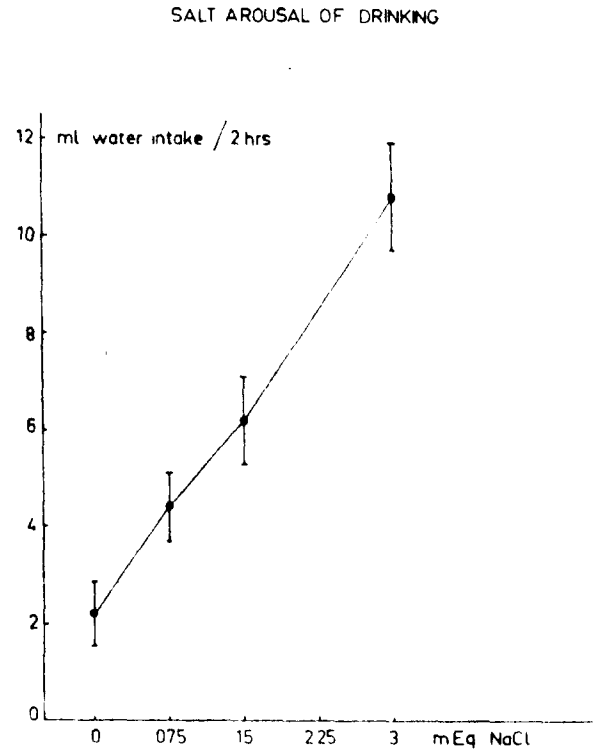

FIG. 2. Effect of graded amounts of subcutaneous administered NaCl on a 2 hr period of water intake in rats. Mean water intake for a group of nine animals.

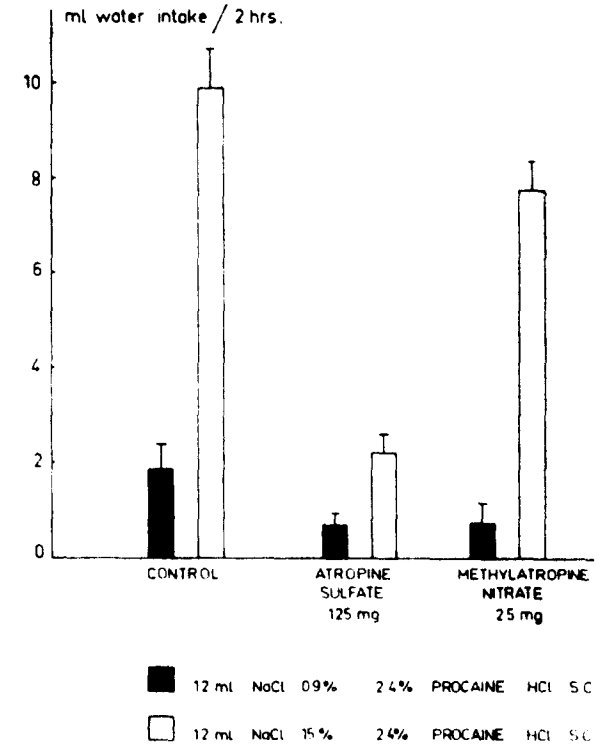

FIG. 3. Effect of a centrally and a peripherally acting anticholinergic agent on the salt arousal of drinking in rats. Eight animals in each group.

Table 1 summarizes results obtained with cholinergic and ganglion blocking agents. Scopolamine which is a more potent centrally acting anticholinergic substance appeared to act superior to atropine in inhibiting the salt arousal of drinking. The ganglion blocking agent mecamylamine which exhibits a marked central activity induced excitation so that the maximum dose was limited to 0.5 mg per 100 g.

TABLE 1

EFFECT OF DRUGS ON THE "SALT AROUSAL OF DRINKING"

CHOLINERGIC AND GANGLION BLOCKING AGENTS				
Treatment			0.9% NaCl	15% NaCl
mg per 100 g IP		N	ml water intake per 2 hr	
Saline	1.80	8	0.9 ± 0.8	8.7 ± 1.4
Atropine	0.50	8	1.0 ± 0.5	8.0 ± 0.9
	1.25	7	0.6 ± 0.3	2.2 ± 0.8
	5.00	8	1.1 ± 0.3	0.9 ± 0.7
Scopolamine	0.03	8	0.5 ± 0.2	5.0 ± 1.4
	0.10	8	0.2 ± 0.1	1.9 ± 0.8
Mecamylamine	0.20	8	0.5 ± 0.1	8.5 ± 1.4
	0.50	10	0.8 ± 0.3	3.3 ± 1.4
Hexamethonium	0.20	8	3.2 ± 0.8	9.8 ± 1.0
	0.50	9	0.4 ± 0.2	4.8 ± 0.6

N = number of observations

In this amount the drug significantly depressed water intake. The quarternary ammonium compound hexamethonium in a similar dose also significantly reduced drinking.

The effect of adrenergic blocking agents on drinking is shown in Table 2. The α-adrenergic blocking agent dibenzyline was administered 18 hr prior to the test in order to prevent severe sedation as occurred when this drug was given a few hours before the salt loading. A slight blocking effect was obtained only when a dose of 3 mg was used. The β-adrenergic blocking drug nethalide was injected at the beginning and one hr later again since it is a short acting drug. A significant depression in water intake was found with the highest dose used. Inderal which is another β-adrenergic blocker also significantly depressed the salt arousal of drinking.

TABLE 2

EFFECT OF DRUGS ON THE "SALT AROUSAL OF DRINKING"

ADRENERGIC BLOCKING AGENTS				
Treatment			0.9% NaCl	15% NaCl
mg per 100 g IP		N	ml water intake per 2 hr	
Saline	1.80	9	1.6 ± 0.5	8.5 ± 0.7
Dibenzyline 18 hr	1.00	8	1.1 ± 1.3	10.1 ± 1.1
	3.00	8	1.2 ± 0.4	6.3 ± 1.5
Nethalide	1.00	9	0.3 ± 0.1	7.4 ± 1.0
	2.00	8	0.4 ± 1.0	3.1 ± 0.5
Inderal	1.00	8	0.3 ± 0.1	6.3 ± 1.0
	3.00	8	0.3 ± 0.1	5.1 ± 0.5

N = number of observations

Results with antihistaminics, antiserotonin and antiparkinson drugs are summarized in Table 3. The antihistaminic drug neobenodine which possesses a weak atropine-like action exhibited a reduction in water intake, only in a dose of 5 mg. The same was true for the two antiparkinson drugs disipal and artane. The antiserotonin agent deseril

TABLE 3

EFFECT OF DRUGS ON THE "SALT AROUSAL OF DRINKING"

ANTIHISTAMINIC, ANTISEROTONIN AND ANTIPARKINSON AGENTS				
Treatment			0.9% NaCl	15% NaCl
mg per 100 g IP		N	ml water intake per 2 hr	
Saline	1.80	7	2.4 ± 0.7	9.5 ± 0.6
Neobenodine	1.00	8	1.6 ± 1.0	9.8 ± 2.8
	5.00	8	0.6 ± 0.2	5.2 ± 0.9
Deseril	0.50	8	0.7 ± 0.4	7.5 ± 0.7
Cyproheptadine	0.25	8	0.9 ± 0.3	9.7 ± 1.0
Disipal	1.00	8	0.7 ± 0.5	8.6 ± 1.1
	5.00	8	0.4 ± 0.2	4.8 ± 0.5
Artane	1.00	8	0.9 ± 0.4	10.3 ± 1.2
	5.00	9	0.6 ± 0.5	4.7 ± 1.8

N = number of observations

was without effect, at least in the dosage used. A higher dose could not be employed because side effects as sedation occurred. The same was found with the antiserotonin-antihistamine compound cyproheptadine. Not more than 0.25 mg per 100 g could be administered. In this dose the drug was ineffective.

TABLE 4

EFFECT OF DRUGS ON THE "SALT AROUSAL OF DRINKING"

MISCELLANEOUS DRUGS				
Treatment			0.9% NaCl	15% NaCl
mg per 100 g IP		N	ml water intake per 2 hr	
Saline	1.80	10	0.9 ± 0.4	10.3 ± 1.2
Cocaine	1.00	8	0.5 ± 0.3	7.5 ± 1.5
	5.00	8	0.2 ± 0.1	2.6 ± 0.7
Amphetamine	0.10	8	0.5 ± 0.2	4.9 ± 2.0
	0.30	8	0.0 ± 0.0	0.1 ± 0.0
Methamphetamine	0.05	8	0.9 ± 0.5	4.9 ± 1.3
	0.15	8	0.4 ± 0.2	1.5 ± 0.7
Chlorpromazine*	0.075	8	0.6 ± 1.0	6.5 ± 0.4
Pitressin tannate*	0.5U	7	0.5 ± 0.2	9.9 ± 1.5

N = number of observations
*Administered subcutaneously

Some miscellaneous drugs were tested as shown in Table 4. The local anaesthetic cocaine significantly depressed water intake.

The two sympathomimetic agents, amphetamine and methamphetamine, completely depressed the water intake in salt loaded rats. These drugs belonged to the most active blocking agents in this respect.

Chlorpromazine injected subcutaneously in a dose which depresses locomotor activity, induced a slight but significant

reduction of the salt arousal of drinking. Pitressin tannate in oil administered subcutaneously 2 hr prior to salt loading failed to affect water consumption of thirsty rats.

The blocking effect of cocaine on the salt arousal of drinking suggested that other local anaesthetics as well might interfere with water intake. Since procaine was used to avoid pain at the site of injection of the hypertonic salt solution it might be that the 28.8 mg used for this purpose could exhibit a thirst blocking effect. The following experiment was performed. Two groups of rats were treated intraperitoneally with the hypertonic salt solution and two groups with saline. Thereafter, these groups were treated subcutaneously either with 2.4% procaine or with saline. It can be seen from Table 5 that this amount of procaine, although it slightly diminished water consumption, did not significantly affect the salt arousal of drinking.

These findings suggest that peripheral mechanisms operate as well in the salt arousal of drinking.

The antiserotonin agent deseril and the antiserotonin-antihistaminic drug cyproheptadine, failed to affect the rate of water intake in the thirsty rats, indicating that neither serotonergic nor histaminergic pathways participate in the process of drinking. Relatively small amounts of these agents were used. Higher doses caused sedation as was the case with chlorpromazine. Non specific depression of drinking may occur under these circumstances. The same holds for other drugs. Since side effects are not always readily recognizable, inhibition of drinking sometimes may be caused by non specific influences.

Although cocaine is structurally related to atropine, its effect on thirst was less pronounced than that of the belladonna alkaloids. Cocaine is a very interesting drug to study

TABLE 5

EFFECT OF SUBCUTANEOUSLY INJECTED PROCAINE HCL ON WATER INTAKE OF RATS TREATED INTRAPERITONEALLY WITH HYPERTONIC SALINE

	WATER INTAKE, ML PER 2 HR			
Intraperitoneal injection	0.9% NaCl	15% NaCl	0.9% NaCl	15% NaCl
Subcutaneous injection	0.9% NaCl		2.4% procaine	
	1.0 ± 0.3	8.7 ± 1.4	2.0 ± 0.5	7.2 ± 0.9

Mean of 7 observations in each group
All substances administered in an amount of 1.2 ml per animal

DISCUSSION

The present experiments show that a variety of agents reduces or blocks the salt arousal of drinking. The most effective drug in this respect was scopolamine, followed by methamphetamine, amphetamine, atropine, the ganglion blocking agents, the β-adrenergic blocking drugs and cocaine.

Montgomery [11] in 1931 was the first to study the influence of atropine on drinking in dogs, but this author failed to find an effect. However, Archdeacon *et al.* [4] found some depression of water intake following the administration of atropine in same species. Adolph [1] working in rats, showed that small amounts of atropine reduced drinking and Schmidt *et al.* [14] demonstrated that atropine depressed drinking in the rat independent of a concomittant reduction in food intake. Recently Stein [15] showed that atropine as well as scopolamine reduced drinking in the rat while their peripherally acting derivatives were without effect.

The postulate that the salt arousal of drinking is mediated by cholinergic stimulation is supported by the blocking effect of atropine, scopolamine and the antiparkinson and antihistaminic drugs with weak anticholinergic activities on the salt arousal of drinking. The fact that the peripherally acting methylatropine was far less active in reducing the water intake of thirsty animals, indicates that the blocking action of the above mentioned substances is located in the CNS.

The peripherally acting quarternary ammonium ganglion blocking compound hexamethonium was as effective in depressing thirst as the tertiary amine mecamylamine, a substance which is more liable to penetrate the brain. since it exhibits amphetamine-like actions [16]. In this respect it is noteworthy that Schmidt [13] reported that cocaine exhibits a marked anorexogenic effect.

The amphetamines belonged to the most effective agents that blocked the salt arousal of drinking in the present experiments. The action of these sympathomimetics on water and food intake is as yet not well understood. Since Grossman [18] has shown that neurochemical adrenergic stimulation elicits eating and simultaneously inhibits drinking in thirsty rats, the effect of the amphetamines on water intake might be explained by a direct sympathomimetic action on the lateral hypothalamic feeding centre. According to this hypothesis the administration of amphetamines should lead to stimulation of food intake. However, food intake has been shown to be depressed in amphetamine treated individuals [3]. It may be however that the stimulating effect of the amphetamines on the food satiety centre overrides a concomittant action on the lateral hypothalamic feeding area. These possibilities are not supported by the findings of Andersson and Larsson [2] who showed that the hunger and thirst inhibiting effects of amphetamine are markedly reduced after prefrontal lobotomy. In addition, Carlsson *et al.* [5] demonstrated that amphetamine failed to decrease the norepinephrine content of the catecholamine terminals in the hypothalamus. These results suggest that the action of the amphetamines in the CNS is not primarily directed towards the hypothalamic areas involved in the regulation of food and water intake.

Since the amphetamines exert their sympathomimetic effects throughout the body, a peripheral action on food

and water intake cannot be excluded. Russek and Pina [12] have shown that peripherally administered epinephrine blocks eating and Miller [9] demonstrated that peripherally injected norepinephrine stops eating as well as drinking. Since the amphetamines act at least partly through the release of catecholamines, it might well be that the locus of the thirst inhibiting action of these compounds is outside the CNS.

The above mentioned results show that whenever a drug contains an intrinsic anticholinergic activity, it reduces drinking in the rat. Since only centrally acting cholinergic blocking agents are effective in this respect, it is clear that cholinergic pathways within the central nervous system are involved. If one accepts that inhibition of the salt arousal of drinking indicates that a substance possesses a central anticholinergic action, the results of the present experiments suggest that thirst produced by hyperosmosis, might be employed to classify centrally acting anticholinergic substances. However, as we have seen, drugs without apparent anticholinergic activities such as the amphetamines and others depress drinking as well. In addition, substances with weak cholinergic blocking effects reduce the salt arousal of drinking in such high dosages that inherent intrinsic activities, directed towards their specific receptors may evoke toxic effects and thus elicit non specific inhibition of drinking. This is a serious drawback for an otherwise relatively easy and rapid method to screen central cholinergic blocking activities of drugs. On the other hand once the anticholinergic action of a substance has been established, the salt arousal of drinking may be employed to determine the locus of action of the drug, its potency and the duration of its action.

REFERENCES

1. Adolph, E. F. Water ingestion and excretion in rats under some chemical influences. *Am. J. Physiol.* **155**: 309–316, 1948.
2. Andersson, B. and S. Larsson. Water and food intake and the inhibitory effect of amphetamine on drinking and eating before and after "prefrontal lobotomy" in dogs. *Acta Physiol. Scand.* **38**: 22–30, 1956.
3. Andersson, B. and S. Larsson. Physiological and pharmacological aspects of the control of hunger and thirst. *Pharmacol. Rev.* **13**: 1–17, 1961.
4. Archdeacon, J. W., M. W. Presnell and C. J. Walton. Effects of atropine on food ingestion and water drinking in dogs. *Am. J. Physiol.* **157**: 149–153, 1949.
5. Carlsson, A., M. Lindqvist, A. Dahlström, K. Fuxe and D. Masuoka. Effects of the amphetamine group on intraneuronal brain amines *in vivo* and *in vitro*. *J. Pharm. Pharmacol.* **17**: 521–524, 1965.
6. Fisher, A. E. and J. N. Coury. Cholinergic tracing of a central neural circuit underlying the thirst drive. *Science, N.Y.* **138**: 872, 1962.
7. Grossman, S. P. Direct adrenergic and cholinergic stimulation of hypothalamic mechanisms. *Am. J. Physiol.* **202**: 872–882, 1962.
8. Grossman, S. P. Some neurochemical aspects of the central regulation of thirst. In: *Thirst in the Regulation of Body Water*, edited by M. J. Wayner. Oxford: Pergamon Press, 1964, pp. 487–514.
9. Miller, N. E. Chemical coding of behavior in the brain. *Science, N.Y.* **148**: 328, 1965.
10. Miller, N. E., K. S. Gottesman and N. Emery. Dose response to carbachol and norepinephrine in rat hypothalamus. *Am. J. Physiol.* **206**: 1384–1388, 1964.
11. Montgomery, M. F. The influence of atropine and pilocarpin on thirst (voluntary ingestion of water). *Am. J. Physiol.* **98**: 35–41, 1931.
12. Russek, M. and S. Pina. Conditioning of adrenaline anorexia. *Nature, Lond.* **193**: 1296, 1962.
13. Schmidt, G. Über die Anorexigene Wirkung des Cocains. *Arch. Int. Pharmacodyn.* **156**: 87–99, 1965.
14. Schmidt, H. Jr., S. J. Moak and W. G. van Meter. Atropine depression of food and water intake in the rat. *Am. J. Physiol.* **192**: 543–545, 1958.
15. Stein, L. Anticholinergic drugs and the central control of thirst. *Science, N.Y.* **139**: 46, 1963.
16. Van Rossum, J. M. and J. A. Th. M. Hurkmans. Mechanisms of action of psychomotor stimulant drugs. *Int. J. Neuropharmacol.* **3**: 227–239, 1964.
17. Wayner, M. J. Jr., J. A. Ross and R. F. Prien. Thirst motivation associated with the salt arousal of drinking. *Am. J. Physiol.* **206**: 369–372, 1964.