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Release of oxytocin contributes to the natriuretic action of aminophylline in rats

MARY F. LOCKETT AND H. L. GWYNNE

Aminophylline, 0·25 to 2·0 mg intramuscularly, caused diuresis, natriuresis and increase in the Na/K of the urine in the 1 hr period after injection into normal hydrated or unhydrated rats. The urinary changes induced by 2 mg aminophylline and by 8 mU oxytocin equated. Salt-maintained adrenalectomized rats were fully sensitive to the diuretic, natriuretic action of aminophylline. The Na/K in the urine decreased at the 2 mg dose level. Hypophysectomy abolished and neurohypophysectomy markedly decreased the diuretic natriuretic action of aminophylline in unanaesthetized rats. Under ethanol-pentobarbitone anaesthesia the diuretic natriuretic action of 0·4 mg aminophylline intravenously lasted 30–40 min in normal rats, and for less than 10 min in neurohypophsectomized rats. The duration of the cardiovascular response to aminophylline was 7–8 min. Thioglycollate-labile oxytocic activity, not detectable in the arterial plasma of control animals, was demonstrable in the arterial plasma of normal rats 8–12 min after 0·5 mg aminophylline intravenously.

THE xanthine diuretics produce both a rise in glomerular filtration rate (GFR) and an increase in the urinary excretion of sodium in man (Howarth, McMichael & Sharpey-Schafer, 1947; Davis & Shock, 1949). Intravenous injections of theophylline-ethylenediamine cause a 35% increase in cardiac output which lasts for about 15 min. This increase in cardiac output is accompanied by a rise in GFR. The rise in GFR cannot, however, explain the total natiuresis. An increase in sodium (Na) clearance is maintained for 50–60 min and clearly outlasts the haemodynamic effects of the drug. Davis & Shock (1949) therefore suggest that the xanthine diuretics may in part produce their renal actions in man directly or indirectly through the neurohypophysis. This hypothesis has now been put to the test in rats.

Experimental

METHODS

Female Wistar rats, 160–212 g, ate a pellet diet (Lockett & Nail, 1965) and drank freely. The fluid supplied to adrenalectomized animals contained 0·7% NaCl: all other rats drank water. Preparatory operations were performed under light pentobarbitone anaesthesia. The techniques used both for adrenalectomy and for total hypophysectomy have been described (Lees, Lockett & Roberts, 1964). Neurohypophysectomy was achieved by placing an electrolytic lesion at the rostral end of the neurohypophysis using a Krieg model Stoetling stereotaxic apparatus for rats, an A.P. co-ordinate of 54·4 mm, with the electrode touching the base of the skull, in the midline. A current of 3 mA was passed for 15 sec. This lesion inflicted no instant injury to the adenohypophysis but caused damage to the vessels of the portal tract. Consequently, the size of the functional adenohypophysis became reduced

From the Department of Pharmacology, The University of Western Australia, Nedlands, Western Australia.

to approximately ½ normal and adrenal weights were found to be ½–⅔rds normal 3 months after operation. Hence an initially dramatic state of diabetes insipidus declined: water intakes were 2–3 times normal when these rats were in use.

LONG TERM EXPERIMENTS

Rats, housed in an air-conditioned room at 24–26°, were accustomed to stomach tubes and handling before use. Each experiment was designed as a series of cross-over tests in which each animal served as its own control. Equal numbers of each treatment were allotted to each day. Tests were made every 3 or 4 days and began with a 2 hr period during which no solid food remained available. At the end of this period each rat in turn received an oral water load equivalent to 2·5% body weight and an injection and was placed in an individual metabolism cage for the collection of all urine formed in the next hour: this collection period was extended to 2 hr for adrenalectomized animals. Since, after training, almost every animal micturated spontaneously when held gently but firmly under restraint for receipt of its water load, bladders were emptied by gentle suprapubic pressure solely to terminate a collection of urine. All cross-over tests which constituted a single experiment were made at a fixed time of day since the urinary excretion of sodium by rats decreases throughout the day (Lees, Lockett & Roberts, 1954). All but two experiments were as described above, during the first hour of a water diuresis. The two remaining experiments differed as follows. Each rat received an oral dose of 3 ml 0·9% NaCl at the start of the 2 hr fast. At the end of the fast an injection was made immediately before the 1 hr period of urine collection, but no water was given.

Oxytocin (Syntocinon, Sandoz Ltd.) and aminophylline (Merck Sharp & Dohme) were dissolved in 0·1 ml 0·9% NaCl, injected subcutaneously and intramuscularly respectively, in long term experiments.

SHORT TERM EXPERIMENTS

Rats were anaesthetized by the intraperitoneal injection of 1·8 mg sodium pentobarbitone and the oral administration of 1·0 ml 12% ethanol, per 100 g. Tracheal and tail vein cannulae were inserted, and a small self-retaining cannula was stitched into the bladder. Thereafter each animal was lightly strapped on its back on a warm table tilted at 45° before an indwelling stomach tube was inserted and strapped to an upright. A steady level of anaesthesia was maintained by administration of 0·5 ml 4% ethanol per 100 g weight, every 20 min, through the stomach tube.

Diureses. When a steady low rate of urine flow had been established (usually within 80 min of induction), 4% creatinine hydrochloride (British Drug Houses Ltd) in 0·9% NaCl was injected subcutaneously, 0·6 ml/100 g wt. Serial 10 min collections of urine began 30 min later and continued for 2 hr (12 periods). One half of the animals in each group received 0·4 mg aminophylline (per rat) in 0·05 ml 0·9% NaCl washed in

with 0·05 ml 0·9% NaCl via the tail vein cannula at the end of the 3rd urine collection; 0·1 ml saline was similarly injected at the end of the 9th collection. The remaining animals received saline at the end of the 3rd and aminophylline at the end of the 6th collection of urine. Samples of venous blood, 0·25 ml were withdrawn from a femoral vein into heparinized syringes immediately before and immediately after the serial collections of urine. Since the concentrations of true creatinine in plasma and whole blood do not differ (Miller & Dubos, 1937) and the decay curve for the plasma concentration of creatinine so administered to rats is exponential from the 30th min (Lippman, 1947; 1948), log concentrations of blood creatinine were plotted against time for each animal. Mid-period plasma concentrations of creatinine were read from these individual curves.

Collection of aortic blood. Eight rats were anaesthetized and equipped with tracheal and tail vein cannulae as described above. Four received 0·1 ml heparinized 0·9% NaCl (2000 units/ml, Evans Medical Ltd) and four 0·4 mg aminophylline in 0·1 ml heparinized saline, intravenously, 30 min after induction of a steady level of anaesthesia. The abdomen was opened in the mid line to permit withdrawal of 4·5 to 5 ml arterial blood from the aorta through a sharpened polythene cannula into a cold nylon syringe during the 12th min after the injection. The plasma was immediately separated by centrifuging at 3,500 rev/min for 30 min at 5°.

EXTRACTION AND ASSAY OF OXYTOCIN FROM PLASMA

The proteins were precipitated from 2 ml samples of rat plasma without delay by addition of 10 volumes of ice cold acetone as described by Ginsburg & Smith (1959). The acetone was removed from the supernatant *in vacuo* at 40°: the resultant cloudy aqueous residue was extracted with 7 volumes of ethyl ether and was cleared of ether at 40° in a stream of air. The final clear aqueous residue was diluted 1:1 with double strength perfusion fluid before assay of the contained oxytocin on an atropinized superfused horn of the rat uterus by the method of Ginsburg & Smith (1959) as thioglycollate labile (Ames, Moore & van Dyke, 1950) uterine stimulant activity.

CHEMICAL DETERMINATIONS

The concentrations of sodium and potassium in urine were measured on an EEL flame photometer. Concentrations of creatinine in whole blood (laked by addition of 0·1 ml blood to 2 ml distilled water) and in urine were determined as described previously for plasma and urine (Davey & Lockett, 1961).

Results

THE INFLUENCE OF INTRAMUSCULAR AMINOPHYLLINE ON THE EXCRETION OF WATER, SODIUM AND POTASSIUM BY UNANAESTHETIZED RATS

Table 1 shows the results of experiments in which the effects of aminophylline on the excretion of water, sodium (Na) and potassium (K) were

TABLE 1. URINARY CHANGES INDUCED BY AMINOPHYLLINE (Am.) DURING WATER DIURESIS IN RATS

Body wt g	Injection	μequiv/100 g rat/1 hr			
		H_2O	Na	K	Na/K
Unoperated					
157 ± 3·21 (16)	Saline	2·16 ± 0·162	14·6 ± 1·77	17·6 ± 2·04	0·97 ± 0·13
	1·0 mg Am.	2·27 ± 0·157	26·0 ± 3·26**	32·6 ± 3·49**	1·01 ± 0·26
	2·0 mg Am.	2·60 ± 0·176**	38·8 ± 4·81**++	42·8 ± 8·34**+	1·15 ± 0·15
155 ± 3·93 (18)	Saline	1·95 ± 0·175	13·2 ± 1·93	17·3 ± 2·12	0·85 ± 0·13
	0·5 mg Am.	1·83 ± 0·112	25·2 ± 2·89**	21·5 ± 3·06	1·36 ± 0·17*
	1·0 mg Am.	2·14 ± 0·182	25·2 ± 4·63**	22·4 ± 3·88	1·31 ± 0·25**
	2·0 mg Am.	1·99 ± 0·179	26·1 ± 3·88**	23·8 ± 3·15*	1·14 ± 0·14*
159 ± 3·42 (18)	Saline	1·92 ± 0·141	15·9 ± 2·97	16·3 ± 2·40	0·96 ± 0·13
	8 mU oxytocin	2·34 ± 0·182*	40·7 ± 5·06**	23·0 ± 3·36*	1·70 ± 0·21**
	2·0 mg Am.	2·26 ± 0·167*	38·7 ± 6·81**	24·5 ± 3·76*	1·52 ± 0·19**
158 ± 3·62 (18)	Saline	1·76 ± 0·17	18·4 ± 1·85	14·4 ± 2·19	1·08 ± 0·18
	8 mU oxytocin	2·09 ± 0·18*	46·1 ± 4·61**	23·6 ± 3·80*	2·11 ± 0·23**
	2·0 mg Am.	2·15 ± 0·13*	35·0 ± 5·07**	16·1 ± 1·92	2·14 ± 0·26**
		mg/rat/1 hr			
		Creatinine	PAH		
159 ± 3·42 (18)	Saline	18·4 ± 1·58	4·25 ± 0·23		
	8 mU oxytocin	18·9 ± 1·49	6·07 ± 0·81*		
	2·0 mg Am.	20·8 ± 1·89	6·90 ± 0·57*		
158 ± 3·62 (18)	Saline	21·1 ± 1·34	4·67 ± 0·36		
	8 mU oxytocin	21·5 ± 1·99	7·47 ± 0·89*		
	2·0 mg Am.	21·8 ± 1·87	7·00 ± 0·77*		
Adrenalectomized salt maintained					
191 ± 5·03 (16)	Saline	1·05 ± 0·11	55·5 ± 2·41	15·4 ± 1·47	2·05 ± 0·23
	1·0 mg Am.	1·37 ± 0·12**	84·2 ± 7·60**	25·5 ± 2·73*	1·95 ± 0·31
	2·0 mg Am.	1·38 ± 0·10**	78·6 ± 4·94**	26·4 ± 1·91**	1·54 ± 0·08*
176 ± 4·74 (14)	Saline	0·98 ± 0·16	50·4 ± 5·12	16·7 ± 2·32	2·98 ± 0·17
	2·0 mg Am.	1·41 ± 0·13**	79·7 ± 6·46**	31·3 ± 4·76**	2·56 ± 0·14*
Neurohypophysectomized					
168·6 ± 3·31 (22)	Saline	2·95 ± 0·17	12·6 ± 2·11	18·8 ± 3·10	0·92 ± 0·22
	1·0 mg Am.	2·90 ± 0·17	15·3 ± 3·08	18·6 ± 2·85	0·98 ± 0·16
	2·0 mg Am.	2·76 ± 0·18	19·8 ± 3·15*	20·8 ± 3·60	1·37 ± 0·31
Hypophysectomized					
164 ± 3·9 (16)	Saline	0·89 ± 0·17	8·7 ± 0·98	6·8 ± 1·20	1·37 ± 0·32
	2·0 mg Am.	0·73 ± 0·15	9·1 ± 2·09	7·9 ± 1·47	1·62 ± 0·33
169 ± 6·0 (16)	Saline	0·83 ± 0·11	10·1 ± 2·73	13·5 ± 2·24	0·96 ± 0·37
	2·0 mg Am.	0·70 ± 0·18	8·2 ± 2·36	11·5 ± 2·26	0·99 ± 0·28
166 ± 3·8 (14)	Saline	0·97 ± 0·16	8·4 ± 0·86	7·0 ± 1·12	1·26 ± 0·29
	2·0 mg Am.	0·78 ± 0·14	9·9 ± 2·17	8·1 ± 1·36	1·59 ± 0·30
176 ± 4·8 (14)	Saline	1·03 ± 0·18	7·9 ± 1·96	11·8 ± 1·80	0·88 ± 0·26
	2·0 mg Am.	1·02 ± 0·21	11·5 ± 3·04	13·9 ± 2·59	1·04 ± 0·21

The values shown are means ± their standard errors. The significance of differences caused by treatments have been examined by *t*-tests in which each animal has served as its own control and is indicated by *: one, $P > 0.95$; two, $P > 0.99$. The number of animals used in each experiment is shown in brackets in the first column.

measured over the first hour of water diuresis. Aminophylline (1 to 2 mg/rat, i.m.) raised the urinary output of Na in all, and increased the excretion of water and K and the urinary Na/K ratio in three of four experiments on normal rats. The urinary changes caused by aminophylline resembled those induced by the subcutaneous injection of 8 mU oxytocin; moreover, both drugs significantly increased the excretion of *p*-aminohippuric acid but not that of creatinine. The inability of these

animals to discriminate between 1 and 2 mg aminophylline is explained by reference to Fig. 1. Maximal renal action is attained in the first hour

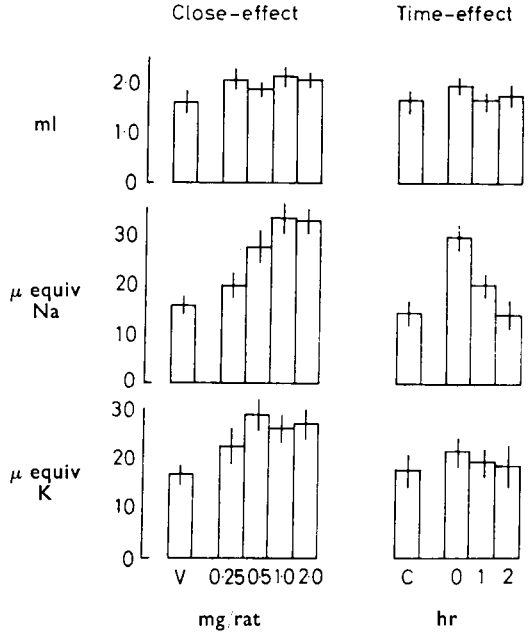

FIG. 1. Dose-effect curve (left) and time-effect curve (right) for the natriuretic action of aminophylline i.m. in water laden unanaesthetized rats. The heights of the columns depict the mean urinary outputs, per 100 g rat, measured over 1 hr period with standard errors of these means. All values were supplied by a single group of 36 rats weighing 161 ± 4·8 g. Left, dose-effect curve: aminophylline i.m. together with an oral water load equivalent to 2·5% body weight at the start of a 1 hr period of urine collection. Abscissae: V, vehicle only, dose of aminophylline i.m. per rat. Right, time-effect curve for 1 mg aminophylline per rat, i.m. Abscissae:—C, no aminophylline, then aminophylline with (0), 1 and 2 hr before water load and subsequent 1 hr period of urine collection. Both experiments were designed as 4-day cross over tests.

after intramuscular administration of this diuretic at a dose level of 1 mg/rat.

Salt-maintained adrenalectomized rats responded to aminophylline by increase in the urinary outputs of water, Na and K: in both experiments, however, the Na/K ratio of the urine was reduced by aminophylline at the 2 mg dose level. By contrast, the urinary excretion of water, Na and K was unaffected by 2 mg aminophylline in totally hypophysectomized animals. Neurohypophysectomized rats, unable to respond to 1 mg aminophylline, showed a small but just significant increase in Na excretion at the 2 mg dose level.

The urinary effects of 2 mg aminophylline in unhydrated animals closely resembled those observed in the same animals when hydrated (Fig. 2).

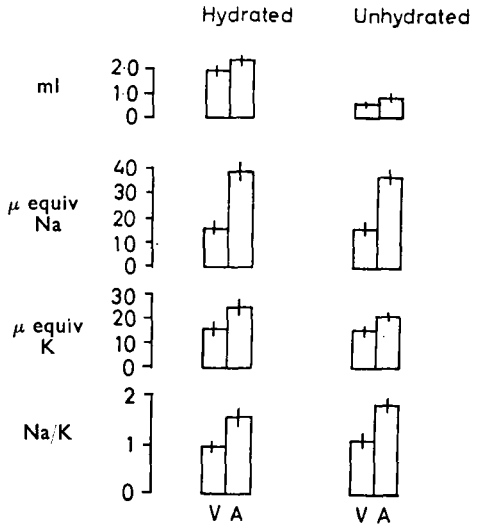

FIG. 2. Comparison of the diuretic effects of 2 mg aminophylline i.m. water laden and in unhydrated rats. The values shown were supplied by a single group of 36 rats, weighing 179 ± 2·8 g, from an experiment designed as a 4-day cross over test. Aminophylline was administered i.m. at the start of a 1 hr period of urine collection with oral water (2·5% body weight) when hydrating, and without water when unhydrated. Values shown are urinary outputs/100 g rat/hr. Ordinates: volume, ml: then μequiv Na and K respectively: Na/K in urine. Abscissae: V, vehicle only: A, aminophylline 2 mg i.m.

THE DIURETIC ACTIONS OF INTRAVENOUS AMINOPHYLLINE IN ANAESTHETIZED NORMAL AND NEUROHYPOPHYSECTOMIZED RATS

The resting rates of urine flow and of glomerular filtration found for normal and for neurohypophysectomized rats under alcohol-pentobarbitone anaesthesia did not differ (Table 2) but the rate of urinary excretion of Na and the Na/K of the urine were significantly greater for the neurohypophysectomized group. Intravenous aminophylline, 0·4 mg/rat, markedly increased ventilation and both the rate and the force of the heart beat in both groups of animals. These respiratory and cardiovascular responses developed within 30 sec of the injection, sustained maximal intensity for 2 to 3 min, then waned. Control levels of ventilation were reached in 15 min and of heart rate by the 7th to 8th min. The cardiovascular actions of the drug are therefore relevant only to the first urine collection made after aminophylline (Table 2). In the first 10 min after intravenous injection, aminophylline produced diuresis, natriuresis and raised the GFR in both normal and neurohypophysectomized animals. However, the diuresis and the increase in GFR shown by the normal animals exceeded that found for the neurohypophysectomized group ($P > 0{\cdot}99$ and $P > 0{\cdot}95$, respectively). Whereas the ratio Na/K in the urine of normal animals rose, this ratio remained unchanged in the urine of the neurohypophysectomized animals because

TABLE 2. CONTRASTS THE URINARY EFFECTS OF 0·4 MG AMINOPHYLLINE, I.V. IN NORMAL AND IN NEUROHYPOPHYSECTOMIZED RATS UNDER PENTOBARBITONE ANAESTHESIA

	Normal	Neurohypophysectomized
body weight in g (number)	163·4 ± 3·91 (6)	170·2 ± 4·90 (6)
Urine: control levels:		
ml/min	0·025 ± 0·007	0·024 ± 0·006
μequiv/min Na	1·60 ± 0·76	5·75 ± 1·35*
K	5·89 ± 1·16	9·33 ± 2·62
Na/K	0·24 ± 0·063	0·65 ± 0·128**
GFR, ml/min	1·36 ± 0·098	1·12 ± 0·059
Urine: 0–10 min after aminophylline		
ml/min	0·135 ± 0·114††	0·064 ● 0·018††
μequiv/min Na	3·83 ± 1·42†	8·22 ± 0·118†
K	7·14 ± 1·07	12·85 ± 2·16†
Na/K	0·54 ± 0·172††	0·62 ± 0·163
GFR, ml/min	1·82 ± 0·236††	1·42 ± 0·128†
Urine: 10–20 min after aminophylline		
ml/min	0·213 ± 0·090††	0·028 ± 0·006
μequiv/min Na	4·61 ± 1·95††	5·61 ± 1·26
K	7·03 ± 2·28	6·72 ± 0·97
Na/K	0·65 ± 0·153††	0·82 ± 0·150
GFR, ml/min	2·07 ± 0·282††	1·10 ± 0·038

The significance of differences between control means supplied by normal and by neurohypophysectomized animals has been examined by group *t*-tests and is indicated by *. Each animal served as its own control in *t*-tests used to assess the significance (†) of changes caused by the drug. Hence significance is indicated both by * and †: one, $P > 0.95$; two, $P > 0.99$.

the excretion of K by the latter group rose in parallel with the excretion of Na.

During the period 10 to 20 min after intraveneous aminophylline normal animals showed a further increase in diuresis, natriuresis, GFR and Na/K in the urine (Table 2). By contrast, resting levels for these parameters were found for the neurohypophysectomized group. Fig. 3

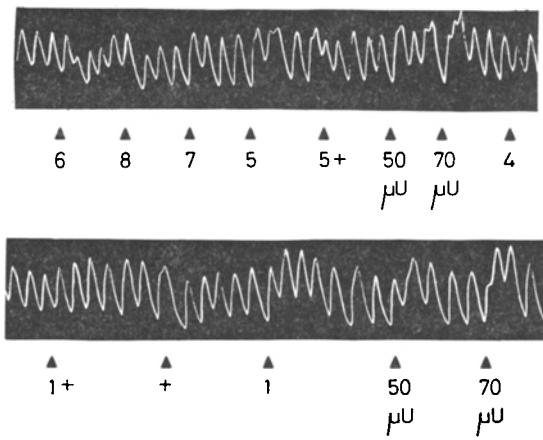

FIG. 3. Trace supplied by a superfused horn of rat uterus, responding, at signals ▲ to the following, in 0·5 ml volume:—6, 8 and 4, extracts equivalent to 2 ml arterial plasma from 3 different control rats. 1, 5 and 7, extracts equivalent to 1 ml arterial plasma withdrawn from 3 different rats 12 min after 0·5 mg aminophylline i.v. The suffix + signifies thioglycollate treatment of the extract, + alone, thioglycollate alone. μU signifies dose of oxytocin. Atropine sulphate, 1 mg/litre, in superfusion fluid.

shows that thioglycollate-labile oxytocic activity was detectable in extracts equivalent to 1 ml aortic plasma withdrawn 12 min after aminophylline (0·5 mg, i.v.) from each of 3 normal rats. By contrast, no oxytocic activity was demonstrable in any of the 3 extracts of 2 ml aortic plasma taken, in parallel, from 3 neurohypophysectomized rats 12 min after aminophylline (0·5 mg i.v.). A similar contrast was found for a fourth pair of extracts prepared from normal and neurohypophysectomized rats' plasma withdrawn 8 min after aminophylline (0·5 mg i.v.).

Discussion

Previous work, especially that of Howarth & others (1947) and Davis & Shock (1949), clearly demonstrated two component mechanisms within the diuretic action of aminophylline in man. The first component was the consequence of the cardiovascular effects which lasted for 15 min after intravenous administration of therapeutic doses. The second component was exposed as a natriuresis which outlasted the haemodynamic action by 40 to 50 min; the mechanism of this natriuresis remained obscure.

The short-lived diuresis, natriuresis and increase in GFR which resulted from intravenous administration of aminophylline to anaesthetized neurohypophysectomized rats (Table 2) synchronized with the cardiac action of the drug. Since these animals, in contrast to normal rats, showed no prolongation of natriuresis beyond the duration of the haemodynamic response, the diuretic action of aminophylline in neurohypophysectomized rats is attributed solely to the cardiovascular effects of the drug. Since the dose of aminophylline, intramuscularly, required to elicit a small increase in the 1 hr output of Na by unanaesthetized neurohypophysectomized rats was twice that maximally effective in normal animals, the cardiovascular component is the lesser contributor to the natriuresis evoked by aminophylline given by this route in this species. The failure of totally hypophysectomized rats to respond to 2 mg aminophylline intramuscularly by natriuresis suggests that the ability of the cardiovascular system to respond to aminophylline is subnormal in these animals. This defect cannot be attributed to withdrawal of corticotrophin and hence to depression of adrenal cortical function since salt-maintained adrenalectomized animals are fully sensitive to the natriuretic actions of aminophylline. The fall in the urinary Na/K which results from administration of 2 mg aminophylline intramuscularly to these adrenalectomized rats is attributed to the hyperventilation caused by the drug and the ease with which these animals develop alkalosis.

The mechanism of the long-lasting component of the natriuresis caused by aminophylline is attributed to the release of oxytocin from the neurohypophysis. The evidence in support of this conclusion is tripartite. First, this phase of the natriuresis is absent in neurohypophysectomized rats. Secondly, the diuretic effects of aminophylline (2 mg i.m.) in normal rats closely resemble those of oxytocin (8 mU s.c.).

Finally, thioglycollate-labile uterine stimulant activity was demonstrated in extracts of 1 ml arterial plasma collected from each of 4 rats 8 to 12 min after intravenous aminophylline but was not found in extracts of 2 ml arterial plasma taken from control animals.

The diuretic actions of aminophylline in water-laden and in unhydrated animals did not differ. Hence it appears that the oxytocin released by aminophylline is not accompanied by functionally significant amounts of antidiuretic hormone. A similar selective release of oxytocin has previously been encountered: the stimulus was low-pitched sound of 50–150 cycles/sec (Ogle & Lockett, 1966; Ogle, 1967).

The direct renal actions of the xanthine diuretics are well known for they have been demonstrated on the perfused dog kidney (Verney & Winton, 1930). Further work is however needed to determine the extent to which the direct effects of aminophylline on the kidney contribute to the therapeutic action of the drug in man.

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