The influence of catecholamines on the diuresis of rats

Group	Treatment	The volume of urine in ml/100 g body wt. after treatment ± S.E.		
		2 h	4 h	24 h
I	Artificial cerebro-spinal fluid (ASCF) (10 µg i.vt.)	0.01 ± 0.00 $n = 30$	0.08 ± 0.02 $n = 30$	1.73 ± 0.21 $n = 27$
II	Dopamine (DA) (100 µg i.vt.)	0.13 ± 0.05 $n = 15$ p I/II < 0.025	0.17 ± 0.06 $n = 15$	1.52 ± 0.25 $n = 15$
III	Pimozide (P) (5 mg/kg i.p.), 2 h later 10 µl of ACSF i.vt.	0 $n = 8$	0 $n = 8$ p I/III < 0.0025	0.81 ± 0.29 $n = 8$ p I/III < 0.025
IV	P (5 mg/kg i.p.) 2 h later DA (100 µg i.vt.)	0.01 ± 0.01 $n = 8$ p II/IV < 0.05	0.01 ± 0.01 $n = 8$ p II/IV < 0.025	1.24 ± 0.26 $n = 7$
V	Noradrenaline (NA) (100 µg i.vt.)	0.23 ± 0.08 $n = 19$ p I/V < 0.01	0.45 ± 0.08 $n = 19$ p I/V < 0.0005	1.28 ± 0.18 $n = 15$ p I/V < 0.025
VI	ACSF i.vt., 10 min later phentolamine (Ph) (100 µg i. vt.)	0 $n = 8$	0.08 ± 0.05 $n = 8$	1.64 ± 0.35 $n = 7$
VII	Ph (100 µg i.vt.), 10 min later NA (100 µg i.vt.)	0 $n = 10$ p V/II < 0.01	0.02 ± 0.02 $n = 10$ p V/VII < 0.0005	1.20 ± 0.10 $n = 10$
VIII	Normetanephrine (100 µg i.vt.)	0.06 ± 0.05 $n = 9$	0.13 ± 0.07 $n = 9$	1.19 ± 0.29 $n = 9$

n = number of rats.

100 g of body weight. Results were elaborated statistically using Students t-test.

Results. DA increased the volume of urine significantly for 2 h after i.vt. injection. Pimozide blocked this effect of DA. Pimozide alone inhibited completely the diuresis during the first 4 h of observation, and inhibited significantly diuresis for 24 h after its application. NA increased significantly the volume of urine excreted 2 and 4, but decreased the diuresis in the total 24 h after i.vt. injection as compared with animals injected i.v.t with ACSF. This effect of NA was blocked by phentolamine for 4 h after its application. Phentolamine and normetanephrine had no significant effect on the diuresis, compared with animals injected with ACSF (Table).

Discussion. Our finding that pimozide, which blocks specifically central dopamine receptors[4], had an antidiuretic action and inhibited diuresis elicited by centrally administered DA, suggests that central dopaminergic receptor activation in our experimental model was responsible for diuretic action of DA. We have observed also a diuretic action of centrally administered NA and a blockade of this effect by phentolamine. Recently it has been suggested that phentolamine has central α-adrenergic blocking activity[5-7]. GUZEK and LEŚNIK[8] have shown that reserpine, which diminishes the storage of brain NA and 5-hydroxytryptamine, decreases the vasopressin content in the hypothalamus and raises the vasopressin content in the blood. Our results suggest that central catecholaminergic receptors may be involved in diuresis regulation.

Résumé. Chez les rats on a observé l'augmentation de la diurèse provoquée par l'injection de la dopamine et de la noradrénaline dans les ventricules latéraux du cerveau. La pimozide, administré dans le péritoine, inhibe l'action de la dopamine. L'action diurétique de la noradrénaline est enrayée après injection intraventriculaire de phentolamine.

H. L. WOLNY, A. PLECH and Z. S. HERMAN

Department of Pharmacology, Biological-Physiological Institute, Silesian School of Medicine, P–41–808 Zabrze 8 (Poland),
11 February 1974.

[4] N.-E. ANDÉN, S. G. BUTCHER, H. CORRODI, K. FUXE, U. UNGERSTEDT, Eur. J. Pharmac. *11*, 303 (1970).
[5] W. DAIRMAN, R. GORDON, S. SPECTOR, A. SJOERDSMA, S. UDENFRIEND, Molec. Pharmac. *4*, 457 (1968).
[6] D. J. GAGNON, K. I. MELVILLE, Int. J. Neuropharmac. *8*, 587 (1969).
[7] Z. KLEINROK, I. ZEBROWSKA-LUPINA, Psychopharmacologia *20*, 384 (1971).
[8] J. W. GUZEK, H. LEŚNIK, Endocrinologie *53*, 189 (1968).

Spontaneous and Testosterone-Induced Motility of Isolated Guinea-Pig Cauda Epididymis

Actually few investigators have directly observed and/or measured epididymal motility, but the employment of indirect techniques suggested 40 years ago the existence of spontaneous contractions in guinea pig epididymis[1-2]. Furthermore, in vivo spontaneous motility of rat epididymis, as well as their response to neurotransmitters[3] and to sex hormones[4], have been observed. The first recordings of spontaneous activity of isolated

human epididymis were obtained by MARTIN et al.[5-6]. In addition, MELIN et al.[7] registered the in vivo spontaneous activity of the distal portion of rabbit cauda epididymis. Recently, HIB[8] reported in vitro, spontaneous motility and responses to oxytocin of the mouse cauda epididymis. In the present study it was explored for the first time the existence and the characteristics of the spontaneous motility of epididymis isolated from guinea-pigs, as well as the effects of testosterone.

Methods. 25 male guinea-pigs weighing from 500 to 700 mg were used. After being sacrificed, by cervical dislocation, the epididymis were isolated. Left and right organs were placed in different Petri dishes containing Krebs-Ringer-bicarbonate solution (KRB) with 11.0 m*M* glucose as the substrate; kept at room temperature and gassed with a mixture of 95% O_2 and 5% CO_2. The KRB solution, composed as follows (m*M*): Na^+ 145; K^+ 6.00; Ca^{2+} 2.5; Mg^{2+} 1.33; Cl^- 126; HCO_3^- 25.3; SO_4^{2-} 1.33; PO_4^{2-} 1.20; had a pH of 7.4 and was kept at a constant temperature of 37 °C throughout the whole experiment. Forthwith the anterior and posterior regions of cauda epididymis were carefully dissected, and immersed in a tissue-bath containing 20 ml of KRB-glucose solution and connected to a tension transducer coupled to an ink-writing oscillograph. After a resting tension of 1 g was applied to the preparations, by means of micrometric device, the cauda epididymis contracted in almost isometric conditions[9]. The preparations were then explored for mechanical activity; the following measurements being made: a) isometric contractile tension (ICT) of phasic cycles; b) tension of tonic contracture (TTC) and c) rate of contractions (RC). The magnitude of epididymal ICT (in mg) was calculated by measuring, at selected moments after isolation, the average amplitude of several consecutive phasic contractions. The TTC (in mg) was the magnitude of the sustained contractile tension developed by the epididymis above its resting or basal tension. The RC represented the number of phasic contractions which occurred during periods of 10 min. These parameters of spontaneous motility were followed for 60 min after isolation.

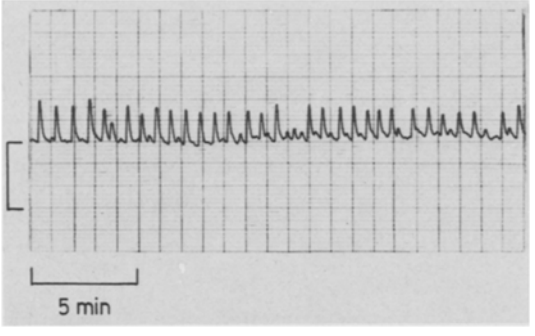

Fig. 1. Original tracing of the spontaneous motility of isolated guinea-pig epididymis.

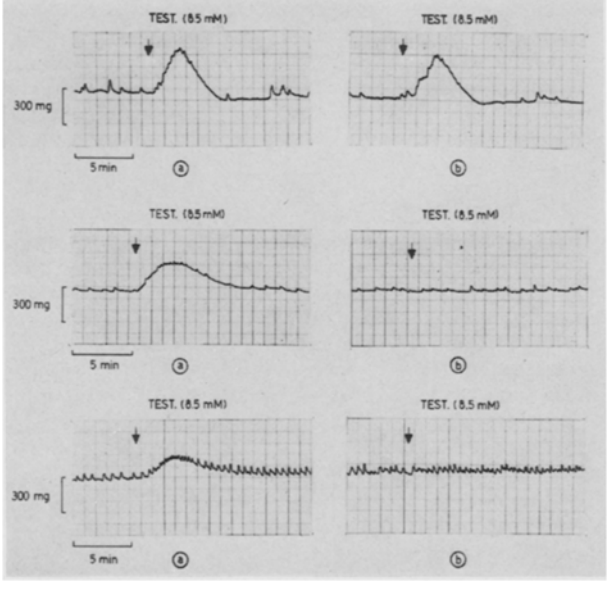

Fig. 2. Original tracings of testosterone-induced motility of isolated guinea-pig epididymis. Effects of atropine, phentolamine and indomethacin. Upper traces: a) Testosterone added (arrow); b) testosterone added (arrow) to a preparation preincubated during 10 min with atropine 0.0057 m*M*. Middle traces: a) Testosterone added (arrow) to a preparation preincubated during 10 min with phentolamine 0.016 m*M*. Lower traces: a) Testosterone added (arrow); b) testosterone added (arrow) to a preparation preincubated during 10 min with indomethacin 0.06 m*M*.

In some cases testosterone (Testosterone Propionate, Gador) at 8.5 or 17.0 m*M* was added between 30 to 60 min after beginning the experiment. Also the influence of indomethacin (Indomethacin, Merck Sharp Dohme) at 0.06 m*M*, phentolamine (Regitine, Ciba) at 0.016 m*M* or atropine (Atropine Sulphate, Sigma) at 0.0057 m*M*, on the effects of testosterone or prostaglandin F_2-α (PGF_2-alpha, Upjohn Co.) at 0.0035 m*M*, was explored. The concentrations of these drugs represent the final ones in the tissue-bath solution. The solvent for testosterone was absolute ethanol in a volume of 0.025 ml. At this level ethanol elicited only a minor depressive influence on epididymal spontaneous contractions. Indomethacin was added in 0.1 *M* sodium phosphate buffer solution. This vehicle proved to be inactive upon epididymal motility. Results were compared using the Student's *t*-test. Differences between means were considered significant when $p = 0.05$ or less.

Results. a) Spontaneous activity of isolated cauda epididymis; effect of testosterone. During the first 60 min after isolation most cauda epididymis (37 out of 50) showed spontaneous motility (Figure 1). There were no differences in the percentage of spontaneously active left and right epididymis (80% and 70% resp.). In most cases the maximal magnitudes of ICT and RC were observed between 20 to 40 min following isolation. No sign of spontaneous epididymal TTC was detected in any of the preparations explored. Mean values of spontaneous ICT, TTC and RC can be seen in the Table. The addition of testosterone elicited a single and distinct contractile response of tonic type (TTC) which had a duration of 8 to 12 min. Over this tonic tension, a superimposed phasic ICT of a magnitude and rate similar to that of the spontaneous activity was also observed

[1] M. C. TOOTHILL and W. C. YOUNG, Anat. Rec. *50*, 95 (1931).
[2] F. A. SIMEONE, Am. J. Physiol. *103*, 582 (1933).
[3] G. MURATORI, Boll. Soc. ital. Biol. sper. *32*, 248 (1956).
[4] P. L. RISLEY, Anat. Rec. *130*, 471 (1958).
[5] T. MARTINS and J. R. VALLE, C. r. Soc. Biol., Paris *129*, 1152 (1938).
[6] T. MARTINS, J. R. VALLE and A. PORTO, J. Urol. *44*, 682 (1940).
[7] P. MELIN, Acta physiol. scand. *79*, 109 (1970).
[8] J. HIB, J. Reprod. Fert. *36*, 191 (1974).
[9] M. F. GIMENO, S. L. BORDA, V. RETTORI, E. BORDA and A. GIMENO, Acta physiol. latinoam. *23*, 363 (1973).

Spontaneous and testosterone-induced motility of isolated guinea-pig epididymis

Spontaneous motility[a]			Testosterone-induced motility[a, b]					
ICT[c] (mg)	RC[d] (C/10 min)	TTC[e] (mg)	ICT[f] (mg)		RC[d] (C/10 min)		TTC[e] (mg)	
			8.5 m*M*	17.0 m*M*	8.5 m*M*	17.0 m*M*	8.5 m*M*	17.0 m*M*
59.0 ± 8.8	13.5 ± 1.5	0	41.0 ± 9.7	56.0 ± 17.3	11.0 ± 1.4	17.0 ± 4.3	205.0 ± 32.0	290 ± 33.0
(37)	(37)	(37)	(10)	(6)	(10)	(6)	(10)	(6)

[a] Mean values ± SEM; figures in the parentheses indicate the number of isolated epididymis studied. [b] Final concentrations in the tissue bath solution. [c] Isometric contractile tension (ICT) of phasic cycles of tension development followed by a complete relaxation down to the point of the tissue resting or basal tension. [d] Rate of contraction (RC) represents the number of phasic contractions (C) in a period of 10 min. [e] Tension of tonic contracture (TTC) developed above the resting or basal tension of the tissue, i.e. the mg developed in a tonic sustained contraction. [f] Testosterone-induced, isometric contractile tension (ICT) of superimposed phasic cycles i.e. a process of tension development followed by a complete relaxation down to the point of TTC elicited by testosterone which is always above the resting or basal tension of tissue.

(Table). The tonic contractile activity elicited by testosterone appeared in most cases after few minutes following its addition. b) Effect of indomethacin, phentolamine or atropine on the contractile response induced by testosterone in cauda epididymis. The repeated addition of testosterone, up to 3 consecutive times, each one preceeded by several washings, had a stimulating influence comparable to that already described in the previous section. Figure 2 shows that this effect of testosterone was blocked by phentolamine ($n = 10$) or indomethacin ($n = 9$), but not by atropine ($n = 6$). On the contrary, neither phentolamine nor indomethacin altered the stimulating influence of $PGF_2\alpha$ on epididymal motility.

Discussion. In the present study the in vitro existence of spontaneous motility in cauda epididymis isolated from guinea pig was demonstrated for the first time. The contractile activity had phasic characteristics, and no spontaneous increments of basal tonic tension were observed.

Numerous papers documented that testosterone has an inhibitory effect in vivo and in vitro over the functioning of the smooth muscle of some organs of the male reproductive tract, such as seminal vesicles, vas deferens and testes[6, 10–14]. On the contrary, in the rat it has been found that testosterone seems to be important for the maintenance of epididymal motility[4–15]. We have observed that this hormone produced a stimulation of epididymal motility characterized by a distinct increase of its tonic tension. The superimposed testosterone-induced ICT was similar in magnitude and frequency to that of the spontaneous epididymal contractions. On the other hand, the stimulating effect of testosterone documented in the present study seems to be indirect; indeed it was abolished by the presence of indomethacin, a well-known inhibitor of the synthesis of prostaglandins[16] and by phentolamine, an alpha adrenergic receptor blocking agent[17]. It must be stressed that the inhibitory influence of these agents upon the effects of testosterone is not non-specific, because both drugs failed to alter epididymal contractile responses to PGF_2-α.

The present experimental results suggest that testosterone stimulates cauda epididymal smooth muscle by means of an indirect mechanism, presumably associated with endogenous noradrenaline and/or prostaglandins. Furthermore, the participation of cholinergic influences does not seem to play any role in the inotropic effect of testosterone[18].

Résumé. Les auteurs décrivent la mobilité spontanée de la partie caudale isolée de l'épididyme du cobaye. Cette mobilité se caractérise par des contractions cycliques régulières et fasciculées. Le testostérone déclanche une contraction soutenue avec des phases cycliques surajoutées, bloquées par la phentolamine ou l'indométhacine, mais non par l'atropine.

M. C. DA SILVA E SOUZA[19], M. F. GIMENO and A. L. GIMENO[20]

Instituto de Neurobiología, Obligado 2490, Buenos Aires 28 (Argentina), 12 March 1974.

[10] W. C. YOUNG and F. A. SIMEONE, Proc. Soc. exp. Biol. Med. *27*, 838 (1930).
[11] T. MARTIN and J. R. VALLE, Endocrinology *25*, 80 (1939).
[12] W. H. KNISELY, J. A. GRAUNT and R. S. BERRY, Am. J. Physiol. *189*, 437 (1957).
[13] R. R. SEELEY, J. L. HARGROVE, R. T. SANDERS and L. ELLIS, Proc. Soc. exp. Biol. Med. *144*, 329 (1973).
[14] J. R. VALLE and A. PORTO, Endocrinology *40*, 308 (1947).
[15] P. L. RISLEY, Anat. Rec. *133*, 329 (1959).
[16] J. R. VANE, Nature New Biol. *231*, 232 (1971).
[17] L. S. GOODMAN and A. GILMAN, in *The Pharmacological Basis of Therapeutics* (The Macmillan Co., 3rd edn. New York, 1965), p. 546.
[18] This work has been supported in part by Grant 6154/74 from the 'Consejo Nacional de Investigaciones Cientificas y Técnicas de la República Argentina'.
[19] Exchange Scholarship Fellow, 'Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina' and 'Conselho Nacional de Pesquisas Brasil'. Maternidade Climerio de Oliveira, Universidade Federal da Bahía, Salvador, Bahía, Brasil.
[20] Member of the 'Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina'.

Effect of 6-Amino-Nicotinamide on the Tolerance of Mice to Hypoxic Hypoxia

In general, normal functioning of the brain and consequently the survival of the whole organism in an atmosphere low or deficient in oxygen are precluded by the high caloric requirements of the mature central nervous organ. Although anaerobic glycolysis is initially stimulated by lack of oxygen[1], the yield of energy is too low to maintain normal cellular metabolism, and anaerobic glycolysis itself will rapidly cease under these condi-