Reduction in fluid secretion by rat testis by drugs that block potassium channels

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The effect of two class III antiarrhythmic drugs (Almokalant, Astra-Hässle and Dofetilide, Pfizer) on fluid secretion by rat testes has been examined. Both drugs reduced fluid secretion, whether this was measured by the amount of rete testis fluid that could be collected 22 h after unilateral efferent duct ligation, or by the difference in mass between the ligated and unligated testes, or by the difference in amount of supernatant fluid after the parenchyma of the ligated and unligated testes had been dispersed and centrifuged. The secretion of potassium, calculated from the amount of potassium in the supernatant fluids from the ligated and unligated testes was also reduced by the drugs, whereas the secretion of androgen-binding protein and inositol was unaffected. The concentration of potassium in the secreted fluid, calculated from the amount and composition of the supernatant fluids, was not affected by treatment of the rats with Almokalant, but was increased in rats treated with Dofetilide and, in these, the concentration of sodium was reduced and that of magnesium and inositol was increased and the concentration of total protein was unaffected. The concentration of androgen-binding protein in secreted fluid was increased in rats treated with Almokalant, while the concentration of testosterone was unaffected. Histological examination of testes from treated rats revealed phagocytosis of stage 19 spermatids in tubules at stages VIII–IX after 2 days, at stages IX–XI after 4 days and at stages VIII–XIV after 7 days, apparently owing to an effect on spermiogenesis. It appears that these drugs interfere with potassium-mediated fluid secretion by the testis, leading to the other changes seen.

Introduction

Class III antiarrhythmic drugs appear to affect the heart by blocking potassium channels (Colatsky and Follmer, 1990). Four types of potassium channel occur in a variety of tissues: voltage-activated, Ca-dependent, ATP-sensitive and receptor-mediated (Muniz *et al.*, 1992, Ferrer *et al.*, 1995), the last type including muscarinic-linked channels (Kurachi, 1995). However, there appears to be little information on their occurrence in the testis, except that voltage-activated channels do not appear to be present (Muniz *et al.*, 1992). During the testing of a new Class III anti-arrhythmic drug, Almokalant (H234/09; Astra-Hässle, Mölndal), it was noticed that the testes of male rats became atrophied after prolonged treatment with the drug. Since potassium transport may be the basis for fluid secretion by the Sertoli cells in the testis (Waite and Gladwell, 1982; Hinton and Setchell, 1993), it is possible that the cause of this atrophy was an interference with potassium and fluid secretion by the Sertoli cells. This possibility was tested and evidence for the involvement of potassium transport in fluid secretion in the

testis was obtained by studying the effect of short-term administration of this drug or another Class III antiarrhythmic drug (Dofetilide, Pfizer, New York) on testicular fluid secretion and composition in rats.

Materials and Methods

Experiment 1

A total of 48 Sprague–Dawley rats, purchased from Alab, Stockholm, weighing between 250 and 350 g at the start of the experiment, were used. They were divided randomly into six groups of eight rats each, and housed four to a cage in an animal house at the Karolinska Hospital. They were fed on diet R36 (Ewos, Stockholm) with water available *ad libitum*. The rats were dosed by gavage using an infant feeding tube each morning between 08:30 h and 09:30 h for 2, 4 or 7 days either with Almokalant (500 µmol kg⁻¹ body mass using a solution of 100 µmol ml⁻¹ in citrate buffer, pH 5.0) or with the same volume of buffer solution. The 2 day groups were done first, the 4 day groups started 5 days later, and the 7 day groups

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after a further 8 days, so that there were intervals of 8 and 10 days between the samplings. On the last day of treatment, between 11:00 h and 13:00 h, each rat was anaesthetized with avertin (8 ml kg⁻¹ body mass i.p. of a solution made by dissolving 1 g 2,2,2-tribromoethanol and 3 ml *tert*-amyl alcohol in 1 ml water, diluting with 4 ml ethanol and then making to 50 ml with saline), and the efferent ducts of the left testis ligated as described by Setchell (1970). Next day, beginning at 09:00 h, that is, 22 h after efferent duct ligation, the rats were anaesthetized with pentobarbitone sodium (50 mg kg⁻¹ body mass i.p.), and the left testis was exposed through a scrotal skin incision. Rete testis fluid (20–150 µl) was collected into a haematocrit tube as described by Setchell and Main (1975); the volume was recorded from the length of the fluid column and the sample diluted with three volumes of saline before centrifugation at 1000 *g* for 5 min to remove the spermatozoa. The right testis was exposed and blood was collected from a testicular vein as described by Galil and Setchell (1988). A second heparinized blood sample was then collected from the posterior vena cava, both testes were removed and the animal was killed. The blood samples were centrifuged at 3000 *g* for 20 min and plasma was separated. The testes were weighed, the capsules were opened and the parenchyma from each testis were separated and weighed. Approximately 100 mg of parenchyma was then removed from one end of the unligated testis and placed into glutaraldehyde fixative for subsequent histology. The remainder of the unligated testis parenchyma and all the ligated testis parenchyma were then extruded through 21 gauge needles with 5 ml syringes into pre-weighed Eppendorf tubes, which were then centrifuged at 10 000 *g* for 15 min to yield a fluid supernatant and cell sediment. The fluid was separated and weighed and the mass of the cell sediment obtained by reweighing the tube. All samples were then stored at –20°C until they were analysed. The supernatant fluids, diluted rete testis fluid (pooled from more than one animal if the volumes were less than the 200 µl required for analysis) and the peripheral blood samples were analysed for potassium by flame photometry at the Clinical Chemistry Laboratory of the Löwenströmska Sjukhuset. Supernatant fluids, testicular venous and peripheral blood samples were analysed for testosterone by radioimmunoassay using a DPC Coat-a-count kit (Diagnostic Products Corporation, Los Angeles) at the Karolinska Hospital. Androgen-binding protein (ABP) in the supernatant fluid was estimated by the method of Ritzen *et al.* (1974).

Fluid secretion was estimated in two ways. Total secreted fluid (that is, tubular and rete fluids) was determined from the mass difference between the two testes plus the amount of RTF collected. Additional or secreted tubular fluid (STF, which has been shown to be largely derived from seminiferous tubule luminal fluid; Setchell *et al.*, 1996) was estimated from the difference between the amounts of supernatant fluid collected from the decapsulated ligated testes (LF) and unligated testes (UF), respectively, after a correction had been made for the amount of unligated testis removed for histology. The amount of potassium and ABP secreted was determined by subtracting the amounts in the supernatant fluid from the unligated testis from the amounts in the fluid from the ligated testis. The concentration in the STF was calculated by dividing the amount secreted by the volume of additional secreted tubular fluid.

The testis samples fixed in glutaraldehyde were processed for light microscopy by embedding in Histo-resin[®], a glycol methacrylate, sectioning at 2 µm and staining with haematoxylin and periodic acid–Schiff. The sections were evaluated under an oil immersion objective (× 60, NA 1.3).

Experiment 2

The procedures adopted in this experiment were essentially the same as in Expt 1, except that instead of Almokalant, the drug Dofetilide (Pfizer) was used at a dosage of 50 µmol kg⁻¹ body mass, using a solution of 7.5 µmol ml⁻¹ in citrate buffer, pH 4.8, and the drug or buffer alone was administered by gavage at 17:00 h each day, with the last dose after the efferent ducts had been ligated. The experiment was performed in two replicates, with half the animals from all groups sampled on one day and the other half on the next day. Rats were DA strain from the Central Animal House of the University of Adelaide and weighed between 151 and 264 g (mean 231 ± 4.2 g) at the start of the trial. They were held in the animal house of the Department of Animal Science, which is maintained at 21°C with lights on at 07:00 h and off at 19:00 h, and fed Joint Stock Ration (Milling Industries, Adelaide) with water available *ad libitum*. Samples were collected as described previously. Potassium, sodium and magnesium were estimated by atomic absorption at Vetlab, Adelaide; inositol was estimated by gas liquid chromatography according to the method of Blakeney *et al.* (1982, 1983); and total protein was estimated by the method of Bradford (1976). The amounts of sodium, magnesium, inositol and protein secreted and their concentrations in the STF were determined as for potassium in Expt 1. No estimations of testosterone or ABP were made in this experiment, because no changes in testosterone were seen in the first experiment and ABP analyses were not available in Adelaide.

Statistical methods

Data were analysed by two-way (drug treatment or control and time of treatment) analysis of variance (Superanova, Abacus Concepts, Berkeley, CA), followed by *t* test for individual groups. The data on concentration differences between ligated and unligated testes were analysed by paired *t* test.

Results

Fluid secretion

Both drugs produced an obvious and highly significant effect on fluid secretion by the testis, whether this was estimated from the difference in mass after efferent duct ligation of the whole testis plus the volume of RTF collected, the difference in the amounts of supernatant fluid between the ligated and unligated testes, or the volume of RTF actually collected (Tables 1 and 2). No attempt was made to adjust the values for differences in testis mass, since there appears to be no correlation between fluid secretion and testis mass in adult rats (Hinton and Setchell, 1993). The effect on the volume of RTF

Table 1. Secretion of fluid, potassium and androgen-binding protein by the testes of Almokalant-treated and control rats (means $\pm$ SEM)

Days of treatment (n)	Almokalant			Control			P versus control
	2 (8)	4 (8)	7 (6)	2 (7)	4 (8)	7 (7)	
Total fluid	338 $\pm$ 30	354 $\pm$ 30**	423 $\pm$ 50	422 $\pm$ 34	481 $\pm$ 27	458 $\pm$ 40	0.003
RTF	41 $\pm$ 7.4*	45 $\pm$ 4.5***	54 $\pm$ 12.3	84 $\pm$ 16	90 $\pm$ 8.8	52 $\pm$ 7.0	0.0009
STF	208 $\pm$ 10.1**	208 $\pm$ 18.5**	277 $\pm$ 21.3	262 $\pm$ 13.4	272 $\pm$ 15.9	334 $\pm$ 32.3 ^{†††}	0.0011
UF	220 $\pm$ 12.4	262 $\pm$ 8.6	303 $\pm$ 8.5	220 $\pm$ 13.7	272 $\pm$ 7.2	320 $\pm$ 13.3 ^{†††}	0.292
K ⁺ secretion	9.7 $\pm$ 0.7*	10.1 $\pm$ 1.1	12.2 $\pm$ 1.6	13.1 $\pm$ 1.5	13.3 $\pm$ 1.1	16.5 $\pm$ 1.7	0.0015
ABP secretion	76.7 $\pm$ 3.9	56.0 $\pm$ 9.8	63.3 $\pm$ 10.8	61.7 $\pm$ 7.9	74.7 $\pm$ 9.2	57.2 $\pm$ 8.0	0.90
Testis mass (mg)	1460 $\pm$ 41	1610 $\pm$ 41	1740 $\pm$ 33	1490 $\pm$ 29	1650 $\pm$ 28	1820 $\pm$ 44 ^{†††}	0.100
Body mass (g)	267 $\pm$ 2.7	322 $\pm$ 3.5	371 $\pm$ 5.0*	275 $\pm$ 2.9	323 $\pm$ 4.5	385 $\pm$ 3.9 ^{†††}	0.376

Total fluid: difference in testis mass (mg) between ligated and unligated testes + volume of rete testis fluid collected (μ l); RTF: volume of rete testis fluid collected (μ l); STF: amount of seminiferous tubule fluid secreted (difference in amount of supernatant fluid between ligated and unligated testes (mg)); UF: amount of supernatant fluid from unligated testis (mg); K⁺ and ABP secretion (nmol and pmol per testis per 22 h, respectively) are calculated as described in the text.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ significantly different from corresponding control by *t* test after ANOVA.

^{†††} $P < 0.001$ significant difference between 2-, 4- and 7-day groups by ANOVA.

Table 2. Secretion of fluid, potassium and other ions and inositol by the testes of Dofetilide-treated and control rats (means $\pm$ SEM)

Days of treatment (n)	Dofetilide			Control			P versus control
	2 (8)	4 (6)	7 (6)	2 (8)	4 (8)	7 (8)	
Total fluid	207 $\pm$ 20***	153 $\pm$ 17***	156 $\pm$ 15***	330 $\pm$ 21	356 $\pm$ 20	259 $\pm$ 14 ^{††}	0.0001
RTF	1.1 $\pm$ 0.8***	0.2 $\pm$ 0.2***	1.5 $\pm$ 0.8***	22.8 $\pm$ 6.8	36.5 $\pm$ 5.2	38.1 $\pm$ 5.1	0.0001
STF	124 $\pm$ 9.3***	105 $\pm$ 12.2***	81 $\pm$ 10.8***	255 $\pm$ 13.0	256 $\pm$ 10.4	180 $\pm$ 8.1 ^{†††}	0.0001
UF	152 $\pm$ 3.7*	150 $\pm$ 11.0**	141 $\pm$ 3.8**	176 $\pm$ 9.2	201 $\pm$ 10.3	182 $\pm$ 13.0	0.0001
K ⁺ secretion	8.3 $\pm$ 1.0*	6.1 $\pm$ 0.9**	4.8 $\pm$ 0.6**	13.3 $\pm$ 1.4	10.3 $\pm$ 0.9	7.8 $\pm$ 0.6 ^{†††}	0.0002
Na ⁺ secretion	10.8 $\pm$ 0.94***	10.0 $\pm$ 0.88***	7.8 $\pm$ 0.86***	28.3 $\pm$ 1.18	27.5 $\pm$ 2.13	20.2 $\pm$ 0.88 ^{†††}	0.0001
Mg ²⁺ secretion	0.37 $\pm$ 0.06*	0.37 $\pm$ 0.10	0.21 $\pm$ 0.04**	0.61 $\pm$ 0.06	0.45 $\pm$ 0.04	0.51 $\pm$ 0.05	0.0005
Inositol secretion	678 $\pm$ 61	591 $\pm$ 30	476 $\pm$ 56	686 $\pm$ 80	549 $\pm$ 44	522 $\pm$ 46 ^{††}	0.94
Testis mass	1003 $\pm$ 24	955 $\pm$ 35**	908 $\pm$ 43	1045 $\pm$ 28	1102 $\pm$ 23	1022 $\pm$ 41	0.0012
Body mass	239 $\pm$ 6.9	215 $\pm$ 12.6	213 $\pm$ 7.4	241 $\pm$ 3.4	228 $\pm$ 5.3	193 $\pm$ 13.2 ^{†††}	0.82

Total fluid: difference in testis mass (mg) between ligated and unligated testes + volume of rete testis fluid collected (μ l); RTF: volume of rete testis fluid collected (μ l); STF: amount of seminiferous tubule fluid secreted (difference in amount of supernatant fluid between ligated and unligated testes (mg)); UF: amount of supernatant fluid from unligated testis (mg); K⁺ secretion (nmol per testis per 22 h) and Na⁺, Mg²⁺ and inositol secretion (nmol per testis per 22 h) are calculated as described in the text.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ significantly different from corresponding control by *t* test after ANOVA.

^{††} $P < 0.01$, ^{†††} $P < 0.001$ significant difference between 2-, 4- and 7-day groups by ANOVA.

which it was possible to collect was much more obvious with Dofetilide than with Almokalant. With Almokalant, the changes in fluid secretion were not associated with any change in the amount of supernatant fluid from the unligated testis, but with Dofetilide, this value was also significantly reduced in the treated animals.

Composition of secreted and other fluids

The reduced fluid secretion was associated with a reduction in the amount of potassium secreted into the tubular fluid in the treated animals in both experiments (Tables 1 and 2), although there were no differences due to treatment with Almokalant in the concentrations of potassium in the additional STF, or the UF or LF (Table 3). Treatment with Almokalant had no effect

on the concentration of potassium in rete testis fluid (12.30 ± 0.22 mmol l⁻¹; $n = 24$). In Expt 2, the concentration of potassium in the STF was similar in the control animals but higher in the Dofetilide-treated animals, and there was a similar difference for the concentrations in UF and LF, with again the LF having a lower concentration than the UF. Insufficient rete testis fluid could be collected from the Dofetilide-treated rats for potassium analysis. In both experiments, the concentration of potassium in the STF was highly correlated with the concentrations in the LF ($P < 0.001$, $r = 0.806$ and 0.763 , respectively, for Expts 1 and 2) but was not significantly correlated with the concentration in the UF ($P = 0.247$ and 0.093 , $r = 0.178$ and 0.260 , respectively).

The secretion of sodium and magnesium was similarly reduced in the Dofetilide-treated rats (Table 2) and, like

Table 3. The composition of additional secreted fluid (STF) and supernatant fluid from unligated and ligated testes (UF and LF) from control rats (C) or rats treated (T) for 2, 4 or 7 days with Almokalant (A) or Dofetilide (D) (means $\pm$ SEM)

	Drug	Group	STF	UF	LF
K (mmol l ⁻¹)	A	C + T	48.6 $\pm$ 1.7***	76.5 $\pm$ 1.14	62.6 $\pm$ 0.98***
	D	C	44.9 $\pm$ 1.98***	84.8 $\pm$ 1.15	63.0 $\pm$ 0.99***
		T	60.1 $\pm$ 2.61 ^{†††}	96.5 $\pm$ 1.38 ^{†††}	81.6 $\pm$ 0.96 ^{†††}
Na (mmol l ⁻¹)	D	C	110.2 $\pm$ 2.6***	66.6 $\pm$ 0.66	90.3 $\pm$ 1.2***
		T	94.6 $\pm$ 3.8 ^{††}	56.9 $\pm$ 1.5 ^{††}	71.4 $\pm$ 1.1***
Mg (mmol l ⁻¹)	D	C	2.29 $\pm$ 0.6***	4.10 $\pm$ 0.12	3.05 $\pm$ 0.065**
		T	3.21 $\pm$ 0.24 ^{†††}	4.60 $\pm$ 0.12 ^{††}	3.96 $\pm$ 0.089 ^{†††}
ABP (fmole mg ⁻¹)	A	C	228 $\pm$ 17***	119 $\pm$ 8.2	177 $\pm$ 9.1***
		T	296 $\pm$ 23 [†]	112 $\pm$ 6.8	198 $\pm$ 10.6**
Protein (mg ml ⁻¹)	D	C	2.7 $\pm$ 2.4***	43.1 $\pm$ 2.2	21.5 $\pm$ 0.6***
		T	-7.3 $\pm$ 7.4	59.2 $\pm$ 4.6 ^{†††}	32.8 $\pm$ 1.4 ^{†††}
Inositol (μ mole ml ⁻¹)	D	C	2.66 $\pm$ 0.12	2.52 $\pm$ 0.12	2.93 $\pm$ 0.21
		T	6.24 $\pm$ 0.60 ^{†††}	2.63 $\pm$ 0.07	4.14 $\pm$ 0.16 ^{†††}
Testosterone (nmol l ⁻¹)	A	C + T	154 $\pm$ 7.6	132 $\pm$ 6.3	142 $\pm$ 4.5

n = 22 for control and treated groups in the Almokalant experiment and *n* = 20 for the control and *n* = 24 for the treated groups in the Dofetilide experiment.

P* < 0.01, *P* < 0.001 significantly different from corresponding UF.

[†]*P* < 0.05, ^{††}*P* < 0.01, ^{†††}*P* < 0.001 significantly different from corresponding control.

C + T: control and treated data were pooled when there were no significant differences between them.

potassium, the concentration of magnesium was higher in the treated animals in the STF, UF and LF, whereas the reverse was the case with sodium. Both sodium and magnesium concentrations in STF were highly correlated with those in LF (*P* < 0.001, *r* = 0.643 and 0.659, respectively), but sodium in STF was not correlated with the concentration in UF (*P* = 0.643, *r* = 0.033), while there was a weak correlation of magnesium concentration in STF with that in UF (*P* = 0.02, *r* = 0.344). The concentrations in vena cava blood plasma of potassium (3.60 $\pm$ 0.076 in Expt 1 and 5.60 $\pm$ 0.087 mmol l⁻¹ in Expt 2), sodium (145 $\pm$ 1.80 mmol l⁻¹) and magnesium (0.714 $\pm$ 0.0090 mmol l⁻¹) were not affected by treatment.

In contrast to the effects on potassium secretion, there was no effect of Almokalant on the amount of ABP secreted into the tubular fluid (Table 1), because a higher concentration in STF fully compensated for the smaller amount of fluid secreted. There were no effects of treatment on the concentrations in UF and LF, while the difference between the two fluids was highly significant (*P* < 0.001). As with the ions studied, the concentration of ABP in STF in Expt 1 was correlated with that in LF, but not with the concentration in UF (*P* < 0.001, *r* = 0.95, and *P* = 0.659, *r* = 0.099, respectively). Similarly, in Expt 2, there was no effect of treatment on the amount of inositol secreted (Table 2), since the concentration in STF compensated for the smaller amount of fluid secreted. A concentration difference was also seen in LF, but not in UF and, again, the concentration in STF was highly correlated for control, treated and pooled data with that in LF (*P* < 0.001, *r* = 0.76, 0.75 and 0.87, respectively) but not with that in UF (*P* = 0.59, 0.62 and 0.46, *r* = 0.12, 0.12 and 0.11, respectively). Treatment with Dofetilide caused increases in the concentration of total protein in supernatant fluid from both unligated and ligated testes. The calculated concentration of protein in STF was not affected by treatment, but it must be said that neither of these values are

significantly different from zero. However, there are difficulties with this method in estimating accurately the concentration of a substance which is present in STF at very much lower concentrations than in the supernatant fluids.

The concentrations of testosterone in STF, UF, LF, testicular venous (34.4 $\pm$ 6.8 nmol l⁻¹) and vena caval blood plasma (14.5 $\pm$ 3.1 nmol l⁻¹) were not affected by treatment with Almokalant. There were significant correlations between testicular venous and vena caval blood plasma (*P* = 0.002, *r* = 0.479) and between STF and LF concentrations (*P* = 0.001, *r* = 0.700), but not between STF and UF concentrations (*P* = 0.98, *r* = 0.004).

Testis and body masses

The testis mass was not significantly affected by treatment with Almokalant (Table 1), but was reduced in the animals treated with Dofetilide (Table 2). However, the animals in the three treatment groups in Expt 1 were studied at different times, and both testis mass and body mass increased with time. The cell fraction made up about 80% of the parenchymal mass, and this proportion was slightly, but significantly, higher (*P* = 0.001) in the Dofetilide-treated rats (82.44 $\pm$ 0.35 versus 80.48 $\pm$ 0.40%). In contrast with earlier observations (Setchell *et al.*, 1976, 1996), probably because of the larger numbers of animals involved in the present study, the mass of the cell fraction was slightly, but significantly, greater in the ligated testes, but neither drug treatment had any effect on this difference (Expt 1: 78.2 $\pm$ 7.4; Expt 2: 85.5 $\pm$ 7.9 mg). There also appeared to be a small, but significant, effect of Almokalant on body mass (Table 1), but the animals in Expt 1 were not individually weighed before the beginning of treatment. In Expt 2, treatment had no effect on body mass (Table 2).

Histology

All control testes appeared normal (Fig. 1a). In the testes of rats treated with either drug, stage 19 spermatids phagocytosed by Sertoli cells were frequently observed, but there was a substantial variation in the severity of the damage among treated animals and also within individual testes. In the Almokalant-treated rats, only four showed limited alterations at 2 days but, after 4 days, all rats showed minor changes, and even after 7 days, there were only a few phagocytosed stage 19 spermatids apparent (Fig. 1c). In the animals treated with Dofetilide, the number of phagocytosed stage 19 spermatids in late stages VIII–IX were somewhat higher than in those treated with Almokalant and, after treatment for 4 days, they were found in tubules in stages IX–XI. After treatment with Dofetilide for 7 days, there were pronounced alterations in some rats (Fig. 1b, d), with many phagocytosed stage 19 spermatids in tubules at stages VIII–I.

Discussion

It is clear from these results that treatment of rats with either of two drugs that block potassium channels caused a profound decrease in fluid secretion by the testes, presumably by affecting the Sertoli cells. This effect was apparent with as little as two daily treatments, and did not appear to become more pronounced with prolonged treatment. Therefore, it would be interesting to examine the effects of single doses of these and similar drugs. The amounts of fluid secreted by the control testes in the present experiment are very similar to those reported earlier (Total fluid: 400–500 mg per testis per 16 h, see Hinton and Setchell, 1993; STF: 270 mg per testis per 16 h, Setchell *et al.*, 1976; 345, 309 and 349 mg per testis per 20 h in three separate experiments, Setchell *et al.*, 1996).

The only other treatments to cause such a profound and sudden decrease in fluid secretion are an intratesticular injection of colchicine or treatment of young rats with di-*n*-pentylphthalate. A dose of 4 µg colchicine produced a 30% decrease within the next 16 h, and 40 µg almost stopped fluid production, measured in the same way as in the present study (Allard *et al.*, 1993). Exposure of isolated tubules to colchicine or an inhibitor of protein synthesis (Brefeldin A) inhibited fluid secretion *in vitro*, as measured by the rate of movement of a luminal oil droplet (Richburg *et al.*, 1994) and, *in vivo*, there was complete inhibition of fluid secretion by the testes beginning 1 h after exposure of immature rats to 2.2 g di-*n*-pentylphthalate kg⁻¹ (Gray and Gangolli, 1986). Other treatments had effects only after prolonged treatment. For example, the addition of 2,5-hexanedione to the drinking water of rats for 5 weeks initially had no effect on fluid secretion, although after about 4 weeks, fluid secretion fell by approximately 50%, and remained depressed for several weeks after the end of treatment (Johnson *et al.*, 1991; Richburg *et al.*, 1994). Both colchicine and 2,5-hexanedione affect microtubule function, but there seems to be no evidence that they affect potassium secretion, or that the drugs used in the present study affect the microtubules. Other drugs that reduced fluid secretion are inhibitors of carbonic anhydrase, with metabolic alkalosis having a similar effect (Setchell and Brown, 1972).

In the Almokalant-treated rats, the decrease in fluid secretion was accompanied by a reduced secretion of potassium, but no change in the concentrations of potassium in the various testicular fluids, suggesting that the drug was acting specifically on potassium transport, and that the effects on fluid movement were secondary. With Dofetilide, potassium secretion was also reduced, but in contrast to the effects of Almokalant, the concentrations of potassium were increased in testicular fluids from the treated rats, suggesting that the drug was having effects on fluid secretion other than those mediated by potassium. While the changes in magnesium transport and concentrations are similar in nature to those for potassium, the amounts of magnesium present in the fluids make it unlikely that magnesium is the driving force behind much of the secretion. Sodium secretion was also reduced by Dofetilide, and the changes in sodium concentrations mirrored those in potassium, indicating that sodium may be moving passively down its electrochemical gradient.

The secretion of neither ABP nor inositol is reduced by treatment with the potassium channel blockers. This finding suggests that the secretion of these two substances by the Sertoli cells (Robinson and Fritz, 1979; Ritzen *et al.*, 1981) is not linked to fluid production. It is a pity that because the two drugs were studied at different times in different countries, different strains of rat were used and both markers could not be measured in both experiments. In phthalate-treated rats, both fluid and ABP secretion by the testes are reduced (Gray and Gangolli, 1986).

The concentrations of potassium and sodium in the additional STF, obtained in the present experiments by the 'difference' technique, are very similar to those found in micropuncture samples of seminiferous tubule fluid (50 and 108 mmol l⁻¹, Tuck *et al.*, 1970; 46 and 110 mmol l⁻¹, Levine and Marsh, 1971; 40 and 135 mmol l⁻¹, Jenkins *et al.*, 1980). However Jenkins *et al.* (1980) also give values for magnesium (1.19 mmol l⁻¹) that are approximately 50% of those reported here. The concentrations of potassium in the RTF are very similar to earlier values for fluid collected by a slightly different technique (14 mmol l⁻¹, Tuck *et al.*, 1970). The concentrations of inositol reported here are also very similar to those for micropuncture fluid (1.83 mmol l⁻¹, Hinton *et al.*, 1981) and for earlier estimates with the difference technique as used in the present study (2.07 mmol l⁻¹, Setchell *et al.*, 1976). The concentration of ABP in STF reported here is also very similar to that found by a similar analytical method for RTF (180 pmol ml⁻¹, Ritzen *et al.*, 1981), but rather higher than those reported in STF collected by micropuncture and a modification of the present technique, analysed by radioimmunoassay (61 and 44 pmol ml⁻¹, Turner *et al.*, 1984). The concentration of total protein in STF was similar to the value reported for samples collected by micropuncture (5.4 mg ml⁻¹, Setchell *et al.*, 1976; Hinton and Keefer, 1983) and is much lower than that reported by Turner *et al.* (1984) (27.5 mg ml⁻¹). The present values are quite variable, and are in fact not significantly different from zero; they are subject to considerable error because we were trying to estimate a low concentration by measuring the difference between two fluids each of which contained high concentrations of protein. However, these results do indicate that Turner's technique suffers from some major defects, and is clearly not as satisfactory as that validated here.

Fig. 1. (a) Section of a control rat testis, showing a normal seminiferous tubule at stage X of the spermatogenic cycle. (b, d) Sections of testes from rats treated for 7 days with Dofetilide; (b) shows parts of two tubules, the upper at stage I and the lower at stage XIII, with extensive phagocytosis of step 19 spermatids; (d) shows a tubule at stage XI, also showing some phagocytosis of step 19 spermatids. (c) Section of a testis of rat treated for 7 days with Almokalant, with a tubule at stage X, showing a small amount of phagocytosis of step 19 spermatids. Scale bar represents 10 μ m.

Nevertheless, Turner *et al.* (1984) found very similar concentrations of testosterone in STF (168 nmol l⁻¹ obtained by micropuncture; 174 nmol l⁻¹ for fluid obtained by a modification of the present technique) as did our earlier studies using the difference technique (Setchell *et al.*, 1978, 174 nmol l⁻¹). However, Davies *et al.* (1978) reported that the concentrations of testosterone in testicular venous blood, as well as from UF and LF, can vary widely among groups of rats.

The histopathological findings of phagocytosed stage 19 spermatids in rats treated with Almokalant or Dofetilide are consistent with an effect on the Sertoli cells, since failure of sperm release in tubules at stage VIII of the cycle is a common finding when these cells are affected (Russell *et al.*, 1990). Similar changes had been observed in a previous study, when rats were given twice the present dose (1000 µmol Almokalant kg⁻¹ for up to 12 days). After 2 and 4 days, there were only minor changes but, after 7 and 12 days, most tubules were affected. In fact, in two rats treated at the higher dose for 7 days, there were severe changes in almost all tubules, and primary spermatocytes and round spermatids were also affected (L. Plöen, unpublished), an effect that could have been mediated through the Sertoli cells. The stages at which phagocytosed spermatids could be seen (tubules at stage VIII after treatment for 2 days, at stage IX–XI after 4 days and at stages VIII–I after 7 days) suggest that the effects of these drugs are quite rapid, and that they act on spermiogenesis. The time from spermiogenesis (end of stage VIII) to stage I is 4.5–5.5 days, and hence the effect may be limited to sperm release. The observation that there are more phagocytosed spermatids after 7 days than at the earlier times suggests that the effect may be progressive, and this is supported by the results of earlier experiments with a higher dose rate for up to 12 days (L. Plöen, unpublished), in which spermatocytes and spermatids were also affected, presumably these changes are also mediated by an effect on the Sertoli cells. The large variation in severity of the effect among animals, and within the testis, suggests that the dose was near the minimum producing morphological changes.

In conclusion, these studies validate the difference technique for estimating the composition of seminiferous tubule fluid and support the suggestion that fluid secretion by the testis is linked to potassium secretion. If the results of the present short-term experiments can be extrapolated to long-term treatment, the atrophy of the testis seen in rats treated with anti-arrhythmic drugs may be explained by an interference by the drug with potassium secretion, and hence fluid secretion.

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