Peculiar response of Brattleboro rats to selenite

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Summary. Brattleboro rats were given, on the 10th day of life, a single high dose of sodium selenite. 90% of Brattleboro heterozygotes responded to the treatment by the formation of cataract whereas homozygotes with diabetes insipidus exhibited no cataract.

In contrast to adult animals, the administration of a single dose of selenite to young Wistar rats causes cataract[1]. One of the initial phases of cataract formation is known to be the permeation of water into the lens due to disturbance of the permeability of eye epithelium membranes[2]. In this context we studied the possibility of evoking a selenium cataract in Brattlebroro rats, especially in individuals with an inborn defect in water metabolism resulting from a complete lack of vasopressin synthesis in the hypothalamus[3].

Materials and methods. A group of Brattleboro rats (49 sucklings) was housed in 5 litters with their mothers till the 30th day of life. On the 10th day of life all rats were given a single dose of 0.02 M Na_2SeO_3 solution s.c. (30 µmoles/kg b.wt). Daily water intake was measured at the age of 40 days. Rats consuming more than 50% of their body weight were considered to be homozygotes (i.e. animals with diabetes insipidus). The occurrence of cataract was evaluated in situ when the rats were 2 months old. All animals were kept under standard laboratory conditions and fed a standard laboratory diet with water ad libitum.

Results and discussion. The group of heterozygotes in our experiments exhibited cataract in 90% of males and 92% of females - a result comparable to that obtained in Wistar rats[1], whereas rats with diabetes insipidus evinced no cataract (table).

Similarly to the situation in Wistar rats[4], the antidiuretic activity cannot be determined in the plasma of either homozygous or heterozygous Brattleboro rats in the 1st 2 weeks of postnatal life. Nevertheless significant differences between the 2 genotypes can be observed in this period: 1. In contrast to homozygotes, antidiuretic activity can be detected in the hypothalamus and neurohypophysis of heterozygotes (analogously to Wistar rats[5]); 2. 14-day-old homozygotes have a higher haematocrit value and plasma osmolarity[6]. 3. Homozygotes have a lower body weight[7].

The data do not permit an unambiquous explanation of why young rats with diabetes insipidus do not respond to selenite administration by cataract formation. The presence of vasopressin in the CNS of young rats may be a necessary condition for evoking selenium cataract. However, we cannot exclude the possibility that rats with a defect of vasopressin synthesis suffer from additional disturbances[8] which affect their response to selenite administration.

Incidence of selenium-induced cataracts in Brattleboro rats

Postnatal day	Number of rats	Weight (g) 40	Water intake (ml/rat/day) 40	Number of cataracts 60
Rats: Heterozygotes				
Males	10	156.3 ± 6.1	23 ± 1	9
Females	12	132.7 ± 3.8	22 ± 1	11
Homozygotes				
Males	17	140.1 ± 4.1	112 ± 4	0
Females	10	125.2 ± 4.0	94 ± 5	0

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Digoxin distribution between plasma and myocardium in hypoxic and non-hypoxic dogs

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Summary. The relationship between plasma and myocardial digoxin concentrations was investigated in hypoxic and non-hypoxic dogs. The results indicate that hypoxic dogs have lower plasma levels, higher myocardial levels, and an altered myocardial distribution when compared to non-hypoxic dogs.

Numerous authors have reported positive correlation between digoxin serum concentrations, myocardial concentrations and therapeutic effect[1-3]. However, others have found that the haemodynamic effects of {^{3}H} digoxin in dogs lagged behind myocardial {^{3}H} levels[4] and that there is heterogeneous distribution of digoxin in the myocardium in acute myocardial infarction[5]. These reports, together with the fact that hypoxia associated with cardiac failure is a contributory factor to digoxin toxicity[6] indicate a need for further investigation into the plasma and myocardial distribution of digoxin during hypoxia.

Materials and methods. 14 greyhounds of both sexes were anesthetized with sodium pentobarbitone (30 mg/kg, i.v.). Following insertion of an endotracheal tube 6 dogs of

weight 22.0±2.0 kg (mean±SEM) and age 23.0±5.4 months, were artificially respired with room air. The remaining 8 dogs (weight 23.6±1.4 kg, age 25.7±3.9 months) were respired with varying O_2/N_2 mixtures to produce a PaO_2 of approximately 60 mmHg. In each animal the left carotid artery was cannulated for both measurement of arterial pressure and collection of arterial blood samples during the experiment. ECG (Lead II) was used to trigger a Nielsen-type heart-rate meter and these parameters together with arterial pressure were monitored on an M19, 6 channel recorder (Devices Ltd, U.K.). Measurement of PaO_2, $PaCO_2$, pH and base excess was carried out during a 90 min stabilization period prior to and 60 min after the administration of digoxin. Following removal of a zero time arterial blood sample, digoxin 0.05 mg/kg (Lanoxin) was administered i.v. over a period of 1-2 min and arterial blood samples removed for analysis of their digoxin content at 5, 10, 15, 20, 30, 40, 50 and 60 min. Immediately after obtaining the 60 min blood sample the animal was killed by bleeding from a cannula previously inserted in the left femoral artery. The heart was removed immediately, and tissue samples taken from the right and left atria, right and left ventricles and the aorta (this serving as a control). Blood gas analyses were performed immediately upon obtaining samples, plasma was stored at −20 °C for no longer than 7 days prior to analysis for digoxin content, and tissue samples were stored overnight at −4 °C prior to analysis. Digoxin was extracted from tissue samples using the method of Coltart et al.[7]. Digoxin levels in tissue and plasma were measured by means of radio-immunoassay (Lanoxitest γkit Burroughs-Wellcome). Statistical treatment of results was by means of Student's t-test (unpaired).

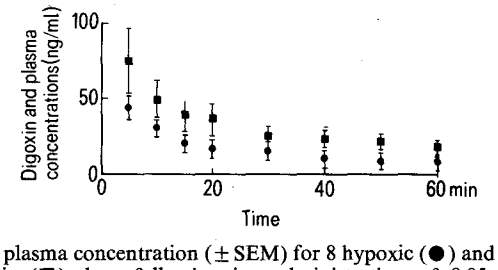
Results and discussion. The arterial oxygen tension in normal patients[8] (those without any form of cardiovascular or respiratory disease) is 102.5±4.7 mmHg (mean±SD). In patients with left ventricular failure with associated pulmonary oedema the arterial PaO_2 may fall to 50 mmHg[9]; the level of hypoxia induced in our study, 56.9±2.1 mmHg corresponds to this group of patients.

Having achieved the required level of oxygenation in each dog, the PaO_2 remained constant during the 90 min stabilization period. During the 60-min study period following administration of digoxin the PaO_2 fell slightly in the dogs ventilated in room air and fell significantly in the hypoxic group ($p < 0.01$; see table 1). This could reflect increased oxygen consumption due to a positive inotropic action on the myocardium[10]. $PaCO_2$, pH and base excess did not change within the groups during the 60 min study period.

The heart rate observed in each group prior to administering digoxin (room air: 188±13.1 beats per min, hypoxic: 205±11.7 beats per min) did not change significantly in the presence of digoxin. Changes in the ECG due to digoxin (lengthening of the P-R interval, shortening of the Q-T interval) were not significantly different between the 2 groups. Arterial pressure although higher in the hypoxic dogs did not differ significantly between the 2 groups.

The Lanoxitest γ radio-immunoassay kit provides a high degree of technical sensitivity at concentrations below 0.5 ng/ml[11,12]. Good agreement has been observed between replicate measurements (within -assay precision) with a coefficient of variation of 0.044% calculated from 30 consecutive assays; the co-efficient of variation for repeatability of assay values (between -assay precision) was 11.7% over 173 values. In the present study all assays were carried out in duplicate, samples were diluted 1:10 to bring them within the range of the assay. The assay[13] was not specific for digoxin but was demonstrated to be capable of detecting digoxigenin and the mono- and bis-digitoxosides. Dihydrodigoxin and dihydrodigoxigenin were not detectable by this assay. In view of the relatively short nature of the experiment and the storage procedures adopted for samples it was considered that significant metabolism did not occur. It is unlikely that the anesthetic affected the assay performance.

Plasma digoxin levels were consistently lower in hypoxic dogs having a value of 46.0±7.5 ng/ml at 5 min, after digoxin administration and falling to 9.2±3.4 ng/ml at 60 min. In dogs respired with room air the corresponding values were 74.2±24.4 ng/ml and 18.8±5.15 ng/ml (see fig.). Although an earlier sample than 5 min might have helped to indicate the difference, if any, in the size of the central compartment between the 2 dogs groups, this (in humans) forms only a small part of the total volume of distribution and could not of itself account for the large differences in plasma concentration seen between the 2 groups. A higher concentration of digoxin would therefore be expected in the peripheral compartment assuming that clearance was similar in both groups. Measurement of digoxin levels in the myocardium supports this concept as the mean digoxin concentration in the myocardium of each chamber of the heart, with the exception of the right atrial appendage, was higher in the hypoxic group (table 2). Whether the differences in measured levels represent differences in the apparent volume of distribution of digoxin



Mean plasma concentration (±SEM) for 8 hypoxic (●) and 6 non-hypoxic (■) dogs following i.v. administration of 0.05 mg/kg digoxin.

Table 1. Values of blood gases, pH and base excess in 8 hypoxic and 6 non-hypoxic dogs prior to and following i.v. administration of 0.05 mg/kg digoxin (mean±SEM)

Blood analysis	Non-hypoxic dogs		Hypoxic dogs	
Initial PaO_2	94.5 mmHg	±6.6	56.9 mmHg	±2.1
Final PaO_2	86.7 mmHg	±6.1	48.6 mmHg	±2.9
Initial $PaCO_2$	36.1 mmHg	±1.5	46.1 mmHg	±5.4
Final $PaCO_2$	34.3 mmHg	±1.5	48.8 mmHg	±7.7
Initial pH	7.355	±0.04	7.28	±0.03
Final pH	7.36	±0.04	7.27	±0.04
Initial base excess	−3.23	±1.4	−5.76	±0.7
Final base excess	−3.12	±1.2	−6.16	±0.7

Table 2. Digoxin concentrations in the myocardial chambers of 6 non-hypoxic and 8 hypoxic dogs 60 min after i.v. administration of 0.05 mg/kg digoxin (mean±SEM)

Digoxin concentrations ng/g (wet weight)	Non-hypoxic	Hypoxic
Left ventricle	46±19	67±22
Right ventricle	33±14	115±41
Right atria	49±20	34±12
Left atria	43±18	65±23

or are indicative of a more rapid distribution in the hypoxic group, is not clear from this study. The apparent myocardium/plasma partition coefficients for each chamber in each dog were calculated as follows:

$$P_{T/P}^{60\,min} = \frac{\text{myocardial concentration in nm/g (at 60 min)}}{\text{plasma concentration in nm/ml (at 60 min)}}$$

The values for $P_{T/P}^{60\,min}$ (right ventricle) were shown to be significantly larger ($p < 0.05$) in the hypoxic group (38.9 ± 13.3 ml/g) compared to the group respired with room air (4.0 ± 1.5 ml/g). Although the mean $P_{T/P}^{60\,min}$ values for the other chambers were 3–5 times greater in the hypoxic group, because of the large variance this difference was not statistically significant.

Comment. This investigation indicates that 1 h after the administration of digoxin i.v.; 1. there is an increase in myocardial digoxin levels in the hypoxic dogs together with a decrease in plasma levels and 2. accummulation of digoxin occurred in the right ventricle of hypoxic dogs. These results warrant further study on the effect of hypoxia on the distribution of digoxin at steady state to see if they explain in the clinical situation the increased risk of toxicity from digoxin in hypoxia[5,6].

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Dopaminergic mechanisms in thiophene-2-aldoxime tremor[1]

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Summary. Tremor induced by thiophene-2-aldoxime is enhanced or reduced in intensity and duration by agents which respectively interfere with or promote dopaminergic tone.

During an investigation of the pharmacology of certain oximes, thiophene-2-carboxaldehyde oxime (thiophene-2-aldoxime, THAO) was found to be a powerful, and chemically novel, centrally-acting tremorigen[2]. Tremor induced by THAO was accompanied by pronounced psychomotor depression and hypothermia, but lacked the rigidity and parasympathetic effects characteristic of tremorine-induced tremor[3]. Of the many classical tremor antagonists studied, d-amphetamine was shown to be the most effective agent in reducing the tremor and reversing the ataxic depression, whereas standard antitremor agents (e.g. atropine) were ineffective[2]. In view of the well-known association of amphetamine with central monoaminergic function[4], we assessed the ability of chemical agents which modify monoaminergic tone to influence the intensity and duration of THAO-induced tremor.

All experiments were performed on male naive albino mice, weighing 25–35 g, at ambient temperatures of

The effect of drug pretreatment on tremor induced by THAO (175 mg/kg)

Pretreatment	Dose (i.p.) mg/kg	Interval before THAO	Mice with tremor/total mice per group	Mean intensity	Duration of tremor (mean ± SE, min)
Saline controls	0.1 ml total	30 min	6/6	(2)	50 ± 5
p-Chlorophenylalanine	150 daily	3 days	6/6	(2)	53 ± 6
5-Hydroxytryptophan	100	60 min	6/6	(2)	56 ± 4
Cinanserin	10	30 min	6/6	(2)	54 ± 4
Reserpine	5	24 h	6/6	(3)	147 ± 7**
alpha-methyl-p-Tyrosine	50 daily	3 days	6/6	(3)	85 ± 2*
Chloropromazine	10	60 min	6/6	(3)	95 ± 1*
Haloperidol	2	45 min	6/6	(3)	110 ± 4**
Ephedrine sulfate	15	45 min	6/6	(2)	11 ± 1**
Chlorimipramine	100	3 h	6/6	(2)	23 ± 3*
Pargyline	50	3 h	6/6	(2)	16 ± 1*
Pargyline	100	4 h	6/6	(1)	15 ± 4*
L-Dopa	150	2 h	6/6	(2)	18 ± 1*
d-Amphetamine sulfate	5	45 min	5/6	(1)	8 ± 2**
d-Amphetamine sulfate	10	45 min	2/6	(1)	3 ± 1**
Clonidine	1	30 min	6/6	(2)	52 ± 9
Apomorphine	3	5 min	6/6	(1)	4 ± 1**

* $p < 0.01$; ** $p < 0.001$ (Student's t-test).