could cause the pore passability of carp red blood cells to be much less than that of human and yellowtail red cells. From this point of view a strong dependence of pore passage time on cell volume is to be expected for carp red cells. However, as seen in figure 1, there seems to be no correlation between them. Furthermore, it was often found that repetition of blood sampling from the same fish caused large but systematic changes in the pore passability of red blood cells without there being any observable changes in mean corpuscular volume. Therefore, the large variation observed in pore passability of carp red blood cells is only partly attributable to differences in cell volume and seems mostly to be due to variability in cellular deformability itself. Loss of blood may stimulate complex responses in the fish which result in the deformability of red blood cells becoming altered considerably. Changes in the population of red blood cells having different deformabilities might also be involved in these modifications.

The pore passage times of carp red blood cells decreased with increasing temperature in a fashion similar to that observed for yellowtail red cells. The increased pore passability of red blood cells at high temperatures will be advantageous for the blood circulation, which must meet greater demands of oxygen and nutrients as a result of the elevated metabolism. The temperature dependence of the blood fluidity through micropores which was observed for carp and yellowtail seems to be consistent with the fact that both fish can live in a wide temperature range.

1 Present address: Department of Physiology, Institute of Applied Electricity, Hokkaido University, Sapporo (Japan).
2 Present address: Research Unit for Comparative Animal Respiration, Bristol University, Woodland Road, Bristol BS8 1UG (England).
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On the role of carbonic anhydrase in the anticonvulsant effects of triethyltin (TET)[1]

S.V. Doctor and D.A. Fox[2]

Division of Toxicology, Department of Pharmacology, University of Texas Medical School, Houston (Texas 77025, USA), 5 October 1981

Summary. Triethyltin (TET) and acetazolamide (ACTZ) both produce marked anticonvulsant effects as evaluated by the maximal electroshock seizure test. However, the mechanism responsible for the anticonvulsant effect of TET and ACTZ is probably not the same since TET does not inhibit the activity of carbonic anhydrase isozyme C in vitro.

Millichap reported that the development of susceptibility to the maximal electroshock seizures (MES) test in the newborn rat is directly related to the levels of the enzyme carbonic anhydrase in the brain[3]. It was suggested that the brain carbonic anhydrase and its catalytic activity are essential for seizure discharge propagation[3]. Further studies with acetazolamide (ACTZ) suggested that low levels of enzyme activity may be sufficient for a focal discharge and the induction of a minor seizure pattern while a high degree of activity was required for a generalized seizure discharge and the induction of a major tonic seizure[4]. In addition to the increased levels of brain carbonic anhydrase, the development of MES susceptibility was related directly to a decrease in brain carbon dioxide levels, inversely to the ratio of extracellular to cellular sodium, and directly to the ratio of cellular to extracellular brain water[5].

Triethyltin (TET) is a member of the organotin class of compounds which are being increasingly used as biocides, preservatives, catalysts, and polymeric stabilizers[6]. TET produces electrolyte alterations which result in white matter edema of the brain and spinal cord, intramyelinic vacuolation and myelin splitting in the rat[7,8]. TET also delays the ontogeny of the MES patterns in developing rats[9] as well as decreases MES responsiveness in adult rats[10]. In order to determine if TET produces its anticonvulsant effect by an inhibition of brain carbonic anhydrase, this study was undertaken to compare the effects of TET and ACTZ on the MES test in adult mice and on a commercially available preparation of carbonic anhydrase C, the isozyme reported to be specific for the nervous system[11].

Outbred male albino mice (25–30 g) were obtained from Timco (Houston, Texas). 5 animals were house per cage and were provided water and Purina chow (Code No. 5008) ad libitum. Animals were maintained on a 12:12 light:dark cycle commencing at 07.00 h. Animals (19–20 mice/group) were injected (i.p.) between 08.00 and 09.00 h each day, in order to avoid circadian rhythm effects, with 0 mg/kg, 100 mg/kg or 200 mg/kg ACTZ (Lederle Laboratories, Pearl River, N.Y.) or 0 mg/kg, 1 mg/kg or 5 mg/kg TET-Br (Alpha Products, Danvers, MA). Additional groups were injected with equimolar doses of stannic bromide ($SnBr_4$), sodium bromide (NaBr) or ethanol to control for the possible ionic effects of tin or bromide or the vehicle effects of ethanol. All animals were dosed with a constant injection volume per body weight (1.0 µl/g b.wt).

All animals were seizure evaluated at 0.5, 4 and 21–24 h following injection. The methods used for eliciting and scoring the MES patterns were modified from techniques described by Woodbury and Davenport[12] and Fox et al.[13]. A 25 mA current was utilized for all mice tested. The indices measured in this study were forelimb flexion, forelimb extension, hindlimb flexion and hindlimb extension. The duration of each phase was determined and then

Table 1. Maximal electroshock seizure (MES) severity in adult mice following acute acetazolamide (ACTZ) or triethyltin (TET) exposure*

Time after exposure (h)	Treatment (mg/kg) ACTZ	TET	Forelimb seizure index** ACTZ	TET	Hindlimb seizure index** ACTZ	TET
0.5	0	0	10.0 ± 0.6^{a}	12.7 ± 0.4^{b}	5.5 ± 0.6	$6.1 \pm 0.4^{i,j}$
	100	1	4.6 ± 0.7^{a}	8.3 ± 0.4^{b}	-	2.9 ± 0.4^{j}
	200	5	2.8 ± 0.5^{a}	4.9 ± 0.6^{b}	-	1.8 ± 1.5^{j}
4	0	0	$13.4 \pm 0.4^{c,d}$	14.4 ± 0.5^{e}	$6.5 \pm 0.4^{k,l}$	6.0 ± 0.4^{m}
	100	1	6.6 ± 0.4^{c}	8.4 ± 0.3^{e}	2.3 ± 0.6^{k}	2.6 ± 0.3^{m}
	200	5	6.1 ± 0.4^{d}	6.1 ± 0.6^{e}	2.2 ± 0.5^{l}	-
21-24	0	0	$13.5 \pm 0.3^{f,g}$	15.3 ± 0.4^{h}	$6.7 \pm 0.3^{n,o}$	7.2 ± 0.5^{p}
	100	1	6.5 ± 0.6^{f}	13.5 ± 0.4^{h}	2.7 ± 0.7^{n}	5.6 ± 0.4^{p}
	200	5	6.9 ± 0.5^{g}	9.5 ± 0.4^{h}	3.5 ± 0.5^{o}	3.1 ± 0.5^{p}

* Values presented are the means ± SE with 7–20 subjects per ACTZ or TET treatment group. Seizure index data was analyzed using an overall unweighted ANOVA. Statistical significance levels reported here are based on posthoc multiple comparisons using Tukey's HDS-test. Groups sharing the same superscript differed at the 0.05 level of significance. ** Forelimb (and hindlimb) seizure index measures were obtained by dividing the duration time of the forelimb (hindlimb) extension by the duration time of the forelimb (hindlimb) flexion for each animal. A decreased seizure index, relative to controls, indicates an anticonvulsant effect.

a forelimb and hindlimb seizure index was determined by dividing the respective extension duration by the flexion duration for each animal (see table 1). A statistical analysis of the seizure index data was performed by an overall unweighted analysis of variance (ANOVA). Statistical significance levels comparing mean values are based on Tukey's honestly significant difference (HSD)-test as described by Winer[14].

Both ACTZ and TET produced profound anticonvulsant effects (table 1). At 0.5 following the administration of ACTZ, greater than 70% of the mice exhibited sub-maximal seizures, (i.e., no hindlimb extension component was present). A similar effect occurred at 4 h in 90% of the mice treated with 5 mg/kg TET. Table 1 shows that an acute treatment with either ACTZ or TET produces anticonvulsant effects lasting greater than 24 h. No differences from controls were observed with $SnBr_4$, NaBr or ethanol.

Human carbonic anhydrase isozyme C was purchased from Worthington Biochemical Co. (Freehold, New Jersey). The carbonic anhydrase assays were performed by a modified procedure[15,16], of Wilbur and Anderson[17]. Ethanol and NaBr were also tested as controls in addition to the TET-Br. ACTZ was tested as a positive control at approximately the same molar concentration as TET-Br. Since a colormetric assay was used, TET-Br was also used as a control in the assay without enzyme to ensure that it did not alter the colormetric end-point without an interaction with the enzyme. The small difference in activity observed with the TET in assays without enzyme was attributable to small amounts of CO_2 present in the inhibitor solution.

The results of this experiment are shown in table 2. No inhibition of carbonic anhydrase was observed using TET-Br, NaBr or ethanol at any of the indicated concentrations. However, ACTZ, at a molar concentration comparable to that of TET-Br, produced approximately 100% inhibition of enzyme activity. The 3×10^{-5} M average concentration reported in body fluids following a 5 mg/kg administration of TET[18] is similar to the lowest level of TET-Br tested in this assay. The acute administration of TET, which produces the physiological concentrations of TET comparable to those used in this experiment, decreases MES severity in adult mice (and rats[10]) to ap-

Table 2. The effects of triethyltin bromide (TET-Br) on the in vitro activity of human carbonic anhydrase isozyme C[a,b]

Treatment	Activity (nmole H^+/min)	
No enzyme	0^{e}	(16)
Enzyme (10 units)	80.0 ± 3.1	(3)
Enzyme (25 units)	198.0 ± 13.3	(3)
Enzyme (50 units)	$310.3 \pm 17.5^{e,f}$	(16)
TET-Br (9.36×10^{-4} M)	$4.7 \pm 1.3^{c,d}$	(6)
TET-Br (1.72×10^{-3} M)	0.8 ± 0.4^{d}	(6)
Enzyme (50 units) + TET-Br (4.68×10^{-5} M)	296.8 ± 18.7	(6)
Enzyme (50 units) + TET-Br (9.36×10^{-4} M)	287.4 ± 18.2	(6)
Enzyme (50 units) + TET-Br (1.72×10^{-3} M)	277.5 ± 39.4	(6)
Enzyme (50 units) + NaBr (1.72×10^{-3} M)	334.4 ± 18.5	(6)
Enzyme (50 units) + NaBr (6.89×10^{-3} M)	277.1 ± 12.8	(6)
Enzyme (50 units) + EtOH (1.71×10^{-5} M)	276.9 ± 15.9	(6)
Enzyme (50 units) + acetazolamide (5.57×10^{-4} M)	$0.8 + 0.6^{e}$	(3)
Enzyme (50 units) + acetazolamide (2.75×10^{-4} M)	0^{f}	(3)

[a] Values presented are the means ± SE. The number of determinations are indicated in the parentheses. Activity data was analyzed using an overall unweighted ANOVA. Statistical significance levels reported here are based on posthoc multiple comparisons using Tukey's HSD. Groups sharing the same superscript differed at the 0.05 level of significance. Treatment groups with 50 units of enzyme were compared independently of the groups without enzyme. [b] The specific activity of the commercially available (Worthington) enzyme was 403 units/mg protein.

proximately the same degree as does ACTZ. However, based on the present biochemical results it appears that the inhibition of carbonic anhydrase isozyme C in vitro is not responsible for the decreased responsiveness to electroshock seizures in animals exposed to TET.

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The significance of species differences in respiratory neurophysiology – the split-brainstem preparation

W.A. Karczewski and H. Gromysz

Department of Neurophysiology, Polish Academy of Sciences Medical Research Center, PL-00-784 Warsaw (Poland), 14 September 1981

Summary. In contrast to the generally accepted view (based upon experiments in cats), a mid-sagittal splitting of the medulla revealed the existence of 2 symmetrical, independent respiratory 'centres' in rabbits and monkeys. The role of species differences is stressed.

It is not clear whether the brainstem neurones that are responsible for the genesis and control of breathing form an integrated, extensively interconnected population, or rather, 2 symmetrical networks active in both halves of the pontomedullary complex and synchronized by decussating fibers. Also, controversy exists over whether there is crossing between the pathways descending from respiratory neurones to phrenic and intercostal motoneurones – and, if there is this crossing, to what extent and at what level it is apparent. Salmoiraghi and Burns[1] have shown in cats that respiratory movements are abolished by a mid-sagittal incision extending from 2 mm caudal to 2 rostral to the obex, and this observation has recently been confirmed (also in cats) by other authors[2,3]. On the other hand, however, a similar incision performed in rabbits by Langendorff et al.[4] in their classical experiments elicited independent contractions of 2 halves of the diaphragm. A similar 'desynchronisation', i.e. independent firing in both phrenic nerves was recently demonstrated (also in rabbits) in this laboratory[5], thus revealing the existence of 2 symmetrical networks of respiratory neurones and their predominantly ipsilateral connections with phrenic motoneurones.

Since the above results were obtained not only in 2 different species but also under different experimental conditions (decerebrate cats[1,2] vs anesthetized rabbits[4,5] and/or a mid-sagittal incision[2,5] vs a mid-sagittal electrolytic lesion[3]), we have undertaken a comparative study with cats, monkeys and rabbits in which – under virtually identical experimental conditions of anesthesia and ventilation – we performed mid-sagittal incisions of the lower brainstem (the 'split-brainstem preparation'[5]) in order to answer the basic question whether species differences or experimental conditions (or both) are responsible for the differences observed.

All animals (14 cats, 3 monkeys *(Cercopithecus callitrichus)* and 7 rabbits) were anesthetized with 0.7 vol.% halothane in an air-oxygen mixture, paralyzed with d-tubocurarine and artificially ventilated. Both vagus nerves were cut in the neck to eliminate the volume-related input from the lungs. Arterial blood pressure and end-tidal CO_2 concentration were continuously monitored. Arterial blood samples were frequently collected for PaO_2, $PaCO_2$ and pH estimations. The electrical activity of both phrenic nerves was simultaneously recorded. An occipital craniotomy was made and the medulla exposed. After control recordings a mid-sagittal incision of the medulla was made with a segment of a razor blade fixed in the holder of a micromanipulator. The blade was lowered towards the base of the skull and this procedure was repeated until a separation of desired length was obtained. The electrical activities of the phrenic nerves, arterial blood pressure and end-tidal CO_2% were continuously recorded during and after surgery. Each experiment was completed by fixing the brainstem in a 10% formaldehyde solution. After 3 days serial sections were made and examined under a microscope to check the localization, extent and completeness of the separation.

Splitting the medulla from about 2 mm caudal to 7 mm rostral to the obex elicited identical effects in normocapnic (and normoxic) monkeys and rabbits: in both species the separation of the 2 halves of the medulla resulted in an immediate 'desynchronization' of inspiratory volleys, i.e. their firing at 2 different rhythms and patterns. On the other hand, in cats, a 'classical'[1] incision from 2 mm caudal to 2 mm rostral to the obex immediately abolished the activity in the phrenic nerves thus confirming the observa-