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Post-exposure therapy with recombinant human BuChE following percutaneous VX challenge in guinea-pigs

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Abstract

Poisoning by nerve agents via the percutaneous (p.c.) route is an issue because the slow absorption of agent could result in poisoning which outlasts the protection provided by conventional pharmacological therapy. The bioscavenger approach is based on the concept of binding nerve agent in the bloodstream, thus preventing nerve agent from reaching the target tissues and inhibiting acetylcholinesterase activity. One bioscavenger that has been extensively studied is human butyrylcholinesterase (huBuChE). Protexia® is a pegylated form of recombinant huBuChE. We used a guinea-pig model of p.c. nerve agent poisoning, using an implanted telemetry system to collect physiological data. Guinea-pigs were poisoned with the nerve agent VX (0.74 mg/kg) (~2.5×LD₅₀). Two hours following VX exposure, Protexia (72 mg/kg) or saline control was administered intramuscularly. All guinea-pigs treated with Protexia (n=8) survived, compared to no survivors in a saline-treated control group (n=8). Survival following VX and Protexia treatment was associated with minimal incapacitation and observable signs of poisoning, and the mitigation or prevention of the detrimental physiological changes (e.g. seizure, bradycardia and hypothermia) observed in control animals. The opportunity for post-exposure treatment may have utility in both civilian and military scenarios, and this is a promising indication for the use of a bioscavenger.

Keywords

Bioscavenger; nerve agent; percutaneous; guinea-pig; VX; medical countermeasures

1. Introduction

Organophosphorous (OP) nerve agents such as VX (O-ethyl-S-[2(di-isopropylamino)ethyl] methyl phosphonothioate), which may be used as chemical warfare agents, are potent

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7. Conflict of Interest Statement Helen Mumford declares no conflict of interest. John K Troyer is employed by PharmAthene, Inc., which manufactured Protexia. The work was carried out by Dstl under Order/ Contract reference BIO/S/1896 Protexia; Work Order 20, funded by Grant Number U01 NS058207 from the National Institute of Neurological Disorders and Stroke (NINDS), a part of the National Institutes of Health (NIH). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the CTSA or NIH.

inhibitors of cholinesterases and act by binding irreversibly to these enzymes. At central and peripheral cholinergic synapses, acetylcholinesterase inhibition leads to neurotransmitter excess, resulting in a range of signs of poisoning including tremor, hypersecretion, status epilepticus and ultimately death. In the case of inhaled OP nerve agent, the onset of signs of poisoning can occur within minutes of exposure. In contrast, following percutaneous (p.c.) nerve agent exposure there is a slower rate of absorption, later onset and longer duration of signs of poisoning (Vale et al., 2007).

The feasibility of using enzymes as bioscavengers for organophosphates has been under investigation for a number of years (Broomfield et al., 1991; Castro et al., 1994; Lenz et al., 2001; Lenz et al., 2005; Cerasoli et al., 2005; Yue-Jin Huang et al., 2008). Human butyrylcholinesterase (huBuChE) purified from human plasma has been shown to provide significant protection against the lethal effects of nerve agents when administered as a pretreatment against a subcutaneous challenge with soman or VX (Lenz et al., 2005) and against inhaled soman (Allon et al., 1998) or sarin vapour (Allon et al., 1998; Saxena et al., 2008). HuBuChE currently has investigational new drug (IND) status in the U.S. as a potential pretreatment drug for use against organophosphate poisoning in humans.

The use of a recombinant form of huBuChE as a post-poisoning therapy has recently been explored in relation to nerve agent poisoning via the p.c. route of exposure in an anaesthetised guinea-pig model, in which Armstrong et al. (2008) reported that recombinant butyrylcholinesterase (rBuChE), delivered intravenously (i.v.) 30 minutes following p.c. VX, decreased the levels of circulating free agent (Armstrong et al., 2008). Some utility has also been demonstrated against p.c. VX poisoning in an anaesthetised swine model (Tenn et al., 2008), although due to the rapid clearance of the un-pegylated rBuChE, repeated administration was required, and more recently in a guinea-pig model (Lenz et al., 2010). In the current study we have used pegylated rBuChE (Protexia®), which has a longer plasma half-life than the un-pegylated material. Protexia also has IND status and is being developed as a pre- and post-exposure therapy for casualties on the battlefield or civilian victims of nerve agent attacks.

Following VX exposure by the p.c. route, we previously showed in a guinea-pig model that there is a progression of clinical and physiological signs of poisoning, including bradycardia, hypothermia, incapacitation and clinical signs such as tremor, salivation and lachrymation (Mumford et al., 2008). A decreased heart rate (bradycardia) appeared to be an early sign of the toxic effects of VX, whereas temperature and observable clinical signs are not good early indicators of percutaneous poisoning (Mumford et al., 2008, 2011). The time to onset of clinical signs, whilst variable, has been shown to be predictive of survival time (Joosen et al., 2008).

Human plasma-derived butyrylcholinesterase has been shown to be effective when administered following p.c. VX poisoning in a guinea-pig model (Mumford et al., 2011). In the current study, we investigated the utility of post-exposure therapy (i.m.) with Protexia administered 2h following a lethal p.c. exposure of VX. Telemetry was used to monitor the effects of the nerve agent and therapy on various physiological indicators (heart rate, EEG, body temperature and locomotor activity).

2. Methods

Experiments were conducted according to the terms and conditions of a project licence issued by the UK Home Office under the Animals (Scientific Procedures) Act 1986.

2.1. Experimental design

This study consisted of two groups of 8 animals each: VX + saline (control) and VX + Protexia. A group size of 8 was chosen based on a Log Rank Test Power Analysis requiring a proportional difference of 0.6 in the number of animals surviving between the control and treated groups, to achieve statistical significance for a power of 70%. Previously-published brain area cholinesterase activity data from naïve weight-matched control animals were included for comparative purposes (Mumford et al 2008).

2.2. Surgery

Animals were surgically prepared according to procedures previously published (Mumford et al., 2010). Briefly, 14 days prior to VX challenge, male Dunkin Hartley guinea-pigs (Harlan Interfauna) were implanted under isoflurane anaesthesia (1.5–2% with O₂ 0.8 l/min and N₂O 0.8 l/min) with telemetry transmitters capable of measuring body temperature, locomotor activity, ECG and EEG (TL11M2-F40-EET, Data Sciences International, St Paul, USA). The transmitter body was inserted into a subcutaneous pocket created by blunt dissection in the interscapular area. The ECG leads were tunnelled subcutaneously to a Lead II position. Two EEG electrodes were inserted to make contact with the dura over the cortex, 4 mm apart, 2 mm to the right of the midline with the anterior hole drilled 4.3 mm posterior of the bregma point. The EEG electrodes were anchored in place with acrylic dental cement (Simplex Rapid, Associated Dental Products, Swindon, UK) before the wound was closed with sutures. Local anaesthetic (Xylocaine 2%) was applied to the wound margins at the time of incision.

Body weight and temperature were recorded daily throughout the experiment. The guinea-pigs were kept in standardised conditions throughout the study, according to Home Office guidelines (room temperature 21°C, humidity 50%). The lights were on from 06:00 to 18:00.

2.3. Nerve agent dosing

VX (O-ethyl-S-[2(di-isopropylamino)ethyl] methyl phosphonothioate) (>98% pure) was synthesized at Dstl and supplied in isopropyl alcohol (IPA). An area of skin on the back, away from the transmitter pocket, was close-clipped. Eighteen hours later VX (22.4 mg/ml) was applied to the prepared area of skin at a dose volume of 0.033 ml/kg giving a dose of 0.740 mg/kg. Pegylated rBuChE (Protexia) was supplied by PharmAthene Inc. at a concentration of 103 mg/ml in buffered saline. Two hours following VX application, either saline (0.9% w/v) or Protexia (72 mg/kg; 0.699 ml/kg) was administered intramuscularly in two equal volume injections into the thigh muscles of the two hind legs. The dose of Protexia was chosen to be stoichiometric to VX (molecular weight 267.4) as follows: $1 \times \text{LD}_{50} \text{ VX} = 296 \mu\text{g/kg}$; equivalent to $1.107 \mu\text{Mole/kg}$ of VX. Molecular weight of Protexia = 65,000, therefore $1 \mu\text{Mole}$ of Protexia = 65mg. Dose required $65\text{mg} \times 1.107 \mu\text{Mole/kg} = 72\text{mg/kg}$.

VX dosing was carried out in a fume cupboard. A 90-minute pre-dosing period was allowed for acclimatization. Following VX dosing, the animals remained in the fume cupboard for 7h under continuous observation. They were then returned to their usual laboratory and observed periodically thereafter. A post-mortem examination was carried out on animals that died during the study and on animals euthanized by anaesthetic overdose at the end of the experiment at 7 days. Tissue samples (plasma, erythrocytes and selected brain areas) were retained for cholinesterase activity determination.

2.4. Cholinesterase analysis

Post-mortem blood samples were collected in tubes containing the anticoagulant EDTA. The samples were centrifuged and separated into red cells and plasma. Haematocrit (packed red

cell volume) was measured in a sub-sample. The cells were washed and made up to the original volume with saline (0.9% w/v). The brains were dissected into the striatum, cortex, cerebellum, hippocampus, midbrain and medulla-pons regions. Tissue samples were stored at -18°C prior to assay. Cholinesterase (ChE) activities were assayed as described previously using acetylthiocholine as a substrate. (Wetherell, 1994). No selective inhibitor was used to determine the acetylcholinesterase activity in the plasma or erythrocytes due to the presence of such a large excess of exogenously-administered BuChE that the AChE specificity could not be reliably guaranteed. Enzyme activities were expressed as μmol acetylthiocholine hydrolysed/min/(ml blood) or per 100mg brain tissue.

2.5. Data analysis

Animals were weighed daily, and post-surgery the weight of the transmitter (8g) was taken into account. Telemetry data were collected using commercial software (Dataquest ATR V4.2, Data Sciences International, USA) with segment duration 60 sec. ECG and EEG were recorded at sampling frequencies of 500 Hz and 250 Hz respectively. Heart rates were derived from the ECG waveform by the computer software and expressed as mean beats per minute for each minute of data. Temperature was measured through a sensor in the implant body and recorded every minute. Continuous telemetry recording was begun at least 24h prior to VX dosing. Control heart rate and temperature were taken as the mean of the last 30-minute predosing period for each individual animal.

Bradycardia (reduced heart rate) was defined for the purposes of this study as a 25% fall from the 30-minute predosing average which was sustained for at least 3 consecutive minutes of valid data. The time of first occurrence of bradycardia following VX was determined on an individual animal basis.

Seizure was determined by visual examination of the EEG trace, either concurrently with observations of animal behaviour or retrospectively by viewing the saved waveforms, and characterised by regular high-amplitude spike activity, either continuous or in intermittent bursts, each of a few seconds duration. Clinical signs of VX poisoning, e.g. tremor, incapacitation, lachrymation and fasciculations, were scored during the observation periods. Posture was classified as normal or incapacitated to a mild, moderate or substantial degree (Wetherell et al., 2002).

Data are presented as mean $\pm$ standard deviation unless otherwise stated. All statistical comparisons were performed using GraphPad Prism Version 5 (GraphPad Software, Inc., USA).

3. Results

The mean bodyweight of the guinea-pigs on the day of surgery ($n=16$) was $318.8 \pm 14.4\text{g}$. On the day of VX dosing, the mean bodyweight was $417 \pm 27.8\text{g}$.

3.1. VX and saline control

The challenge dose of VX (0.74 mg/kg) was selected as one that would kill all animals in the absence of effective medical countermeasures and, as expected, all saline-treated animals died within 48h (Figure 1). There was a progression of adverse physiological signs leading to death, including bradycardia, hypothermia and seizure (Figure 2). In the saline-treated animals, two out of eight animals showed seizure, the onset of which was between 5h 40m and 7h prior to death.

At the time of saline administration (2 h following VX), no animal in this control group showed observable signs of systemic cholinergic poisoning, but most animals were showing signs of agitation and sensitivity to noise.

Animals which died during the observation period had blood and tissues removed for cholinesterase determination, heart and lungs removed and weighed and a post-mortem examination was carried out at that time. Animals that died during the night were examined the following day, but no samples were retained from those animals for cholinesterase analysis or heart/lung weight. Post-mortem examination revealed abnormalities in the gastrointestinal tract and haemorrhagic lungs in a number of animals (Table 1).

3.2. VX and Protexia at 2h

Therapy with Protexia (72 mg/kg) administered by the intramuscular route 2h following VX (0.74 mg/kg) prevented mortality in 8 out of 8 animals (Figure 1). The survival of the two groups was significantly different ($p < 0.0002$; Fisher exact test.)

At the time of therapy administration 7 out of 8 animals did not show observable signs of systemic poisoning. One animal, however, had salivation which was observed when it was picked up for therapy administration. This animal subsequently developed seizure activity, which continued intermittently up to 54h, after which time further physiological recordings were not made, however the presence of unresolved mild intermittent seizure did not prevent the animal from moving, eating and drinking normally, as evidenced by the subsequent weight gain. Figure 3 illustrates representative data from two individual animals; in the first case (Figure 3a) incapacitation was not observed at any time, although piloerection was observed from 5–8h. Two animals developed a substantial and prolonged period of hypothermia, one of which is shown in Figure 3b; this subsequently reversed in both cases and body temperatures returned to within the normal ranges. Both animals survived to the end of the experiment at 7 days.

At 24h all 8 animals had lost weight; an average of 30g or 9% of dosing weight. Six out of eight animals had put on weight at 48h and all except one animal had exceeded their dosing weight by the end of the experiment at day 7.

All 8 animals were euthanized on day 7 by an overdose of anaesthetic; blood and tissue samples were removed for cholinesterase determinations. A post-mortem examination was carried out on all animals at this time and the lungs and gastrointestinal tract of animals in this group appeared normal on visual examination (Table 1).

3.3. Cholinesterase activity

Blood (plasma and erythrocytes) and tissue from six brain areas were analysed for cholinesterase activity. The results are summarised in Table 2. A previously-published group of naïve weight-matched control animals were used to provide data for the normal activity (Mumford et al 2008). Due to the low numbers in the VX + saline group, it was not possible to compare statistically between this group and the control group. However, the cholinesterase activity in all tissues from these two animals was lower than controls indicating that the VX had inhibited the cholinesterase. These results are entirely as expected.

Animals that received VX + Protexia were sampled 7 days following VX poisoning. The cholinesterase activity was significantly lower in the hippocampus, midbrain, pons and cerebellum of the Protexia-treated animals than that of the corresponding naïve weight-matched controls (Table 2). In both plasma and erythrocytes the activities were significantly

higher than control, although there was a large variation in the plasma cholinesterase activity.

4. Discussion and Conclusions

Protexia (72 mg/kg; 0.699 ml/kg) administered 2 h following a supralethal dose of VX (0.740mg/kg) was effective at preventing nerve agent-induced lethality in 100% of cases. This is a significant result that expands previous findings (Tenn et al., 2008; Lenz et al., 2010), demonstrates the efficacy of Protexia in the guinea-pig and broadens the understanding of its physiological effects by using telemetric methods to monitor heart rate, temperature and activity in conscious animals over extended periods.

The adverse physiological changes seen following VX (bradycardia, hypothermia and seizure) were minimised or prevented in animals that received Protexia. Although some animals did have periods of reduced temperature and heart rate, these were less profound than those observed in saline-treated animals, and the Protexia group all recovered. Some animals that received Protexia did not show any observable signs of systemic cholinergic poisoning, (e.g. Fig 3 left) and their heart rates and body temperatures remained within the normal range.

For acute nerve agent poisoning, a stoichiometric relationship between scavenger and inhibitor was previously thought to be required for protection. The dose of Protexia used in this study was calculated to be stoichiometric to 296µg/kg of VX whereas the actual challenge dose of VX used was 740µg/kg. Although the Protexia dose used is lower than the stoichiometric dose, a large enough proportion of the applied VX must have been bound in the circulation to overcome the toxic effects, although in this study free agent levels in the blood were not measured. Following percutaneous poisoning in particular, only a small fraction of the applied VX enters the systemic circulation. Other workers have shown that 7 h following p.c. VX application the bioavailability of agent in blood was only 2.5% of the applied dose, albeit with a significant further increase expected after that time (van der Schans et al, 2003). The bioavailability of pegylated rBuChE following i.m. administration in guinea-pigs has been determined to be approx. 46%, with a plasma half-life of approx. 44 h (Yue-Jin Huang et al., 2007).

Intussusception was associated with the deaths of 4/8 animals in the saline-treated group. Intussusception is a consequence of nerve agent poisoning that has been previously reported following acute soman intoxication (Wetherell et al., 2007) and has also been observed in previous studies following VX poisoning via the percutaneous route (Mumford et al., 2011) and via microinstillation (Katos et al., 2007). Full intussusception was observed in two saline-treated guinea-pigs, and a further two animals had changes in the gastrointestinal tract consistent with the initial stages of intussusception; one further animal had abnormal changes in the lower GI tract which were not judged to be the start of intussusception. Intussusception as a consequence of nerve agent poisoning generally develops over the course of several hours, typically between 6 and 24 h in guinea-pigs (Wetherell et al., 2007). Some animals in the saline-treated group in the present study died from other effects of VX, before pathological signs consistent with intussusception, if present, would have developed.

At this dose of VX, not all guinea-pig would develop seizure, even in the absence of effective medical countermeasures. The seizure incidence in the saline control group in the present study (2/8) was comparable to that seen previously (Mumford et al, 2011). Only one Protexia-treated animal developed seizure, but this was intermittent and did not appear to have been behaviourally detrimental. There could be long-term histopathological changes as a result of prolonged uncontrolled seizure activity (Taysse et al, 2006), but that was not measured in this study.

Cholinesterase activity was measured using acetylthiocholine as the substrate. This is therefore a non-selective measure of total cholinesterase activity. The brain area cholinesterase activities from the Protexia-treated animals were lower than those in the naïve weight-matched animals. It cannot be definitively stated that administration of Protexia protected brain cholinesterase: as the Protexia was administered 2h post VX challenge, inhibition of the cholinesterase by VX may have already been substantial at this point. In addition, the cholinesterase activity was measured following 7 days of a dynamic interaction between the endogenous cholinesterase inhibited by the VX, the exogenous huBuChE and de novo production of cholinesterase initiated at the time of poisoning. Interestingly, the erythrocyte cholinesterase levels in animals administered Protexia were elevated above controls after 7 days. This is in agreement with our previous findings (Mumford et al 2011) and the explanation for this is not clear, but it may reflect compensatory up-regulation of endogenous enzyme in the intervening 6 days (Dorandeu et al, 2008). We cannot rule out some residual BuChE activity in the red cells following the washing procedure, but the use of specific inhibitors is precluded due to the large excess of exogenously-administered BuChE present. The elevated plasma cholinesterase levels (over 7-fold higher than controls) measured after 7 days indicate a long retention time of the exogenously-administered recombinant huBuChE.

In the current study, therapy was administered at 2h post-poisoning. This was chosen as a time at which observable signs of systemic poisoning would not have commenced, and in all but one animal this was the case. In a more realistic scenario, therapy might not be administered until poisoning was evident. In that case, given the available pharmacokinetic data, Protexia given via the intramuscular route may not be completely effective in protecting against the nerve agent poisoning because the circulating levels of nerve agent would have already reached a toxicologically significant level in plasma and in the target tissues (in order to produce cholinergic signs of poisoning). It has been demonstrated (Lenz et al, 2010) that following i.m. administration there is a substantial time lag before peak plasma concentration of the protein would be achieved, namely a T_{max} of 4.2h for unpegylated rBuChE and 20h for plasma-derived huBuChE. Therapy delivered by the i.v. route would be expected to achieve improved protection because of the much shorter time to peak plasma concentration and the improved bioavailability of the scavenger enzyme (Mumford et al, 2011; Yue-Jin Huang et al., 2007).

In conclusion, the use of recombinant huBuChE (e.g. Protexia) as a therapeutic medical countermeasure is a promising treatment strategy against p.c. nerve agent poisoning. This study has demonstrated that bioscavengers can be beneficial when given as a post-exposure therapy. This suggests that it may be useful in both civilian and military exposure scenarios. In contrast to the military situation, some key features of therapy for nerve agent poisoning in a civilian setting are that neither pretreatment nor immediate self-medication are an option. Following a nerve agent release in a civilian context, the delay between exposure of casualties to nerve agent and the ability of medical first responders to deliver treatment will be influenced by several variables, including the timely identification of the intoxicating agent, the time taken to acquire the necessary therapeutic drugs from stockpiles and load them into emergency vehicles, the time taken to arrive at the incident and don protective equipment and the time taken for first responders to gain access to casualties at the scene of the release (Sheridan, R.D., pers. comm.). In this context, relatively little is known of the effectiveness of delayed pharmacological interventions in victims of nerve agent poisoning.

It is recommended that future work investigates the consequences of administration of therapy triggered by observed signs of systemic cholinesterase poisoning and the effect of route of administration on the outcome. For advanced development, it is recommended that

this material be tested in an additional animal species (e.g. the minipig) to increase confidence in extrapolation to humans.

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Highlights

- OP nerve agents like VX may be toxic through skin absorption
- We gave post-exposure therapy of bioscavenger (Protexia) to VX- poisoned guinea-pigs
- This therapy was 100% effective when administered 2h after VX poisoning

Figure 1.

Survival curves of animals treated with VX (0.74 mg/kg) and therapy at 2h with either saline or Protexia® (72 mg/kg). There was a significant difference in survival outcome (Fisher exact test; $p=0.0002$).

Figure 2.

Representative examples of the progression of physiological changes in animals receiving VX (0.74 mg/kg p.c.) at time 0 and saline (i.m.) at 2 hours. The time of therapy administration is denoted by a vertical arrow. Lower (black) lines: heart rate; upper (grey) lines: body temperature (right axis). The horizontal bar at the top of the figures indicates, where present, a period of seizure activity. (a) Mild incapacitation was observed from 4h30, worsening through moderate to substantial incapacitation at 6h 15. Lachrymation was observed from 5h 30 and the animal became hypoxic and died at 6h 40. (b) Incapacitation commenced at the onset of seizure activity at 7h; following this tremor and fasciculations were evident until the end of the observation period at 7h 30. The animal died at 14h 08.

Figure 3. Representative examples of the progression of physiological changes in animals receiving VX (0.74 mg/kg p.c.) at time 0 and Protexia® (72 mg/kg i.m.) at 2 hours. The time of therapy administration is denoted by a vertical arrow. Lower (black) lines: heart rate; upper (grey) lines: body temperature (right axis). (a) Piloerection was observed from 5–8h but there was no incapacitation during the periods of observation (0–8h; 22–31h, 48h). (b) This animal showed some salivation at around 5h and was mildly incapacitated from 5h30 to 8h. At the next observation period, beginning at 23h, the posture was normal and there were no evident cholinergic signs of poisoning.

Table 1

Incidence of selected pathological signs following VX (p.c. 0.74 mg/kg) and therapy (i.m.) with either saline (0.699 ml/kg) or Protexia (72mg/kg) 2h following VX administration.

Treatment	Time of therapy administration	7-day survival	Seizure incidence	Abnormal lungs at post mortem	Heart + lung wet weight (% of body weight)	Intussusception or jejunal spiralling	Time of death	Time to onset of bradycardia
VX + saline	2h	0/8	2/8	8/8	-	4/8	11.90 h	5.7 h
					-		36.53 h	12.9 h
					-		20.35 h	14.3 h
					-		8.98 h	4.4 h
					1.369		4.17 h	2.8 h
					-		14.13 h	5.5 h
					1.375		6.67 h	4.2 h
					-		18.4 h	3.3 h
VX + Protexia	2h	8/8	1/8	0/8	1.34 ± 0.12	0/8	Euthanized @ 7 days	n/o
								n/o
								n/o
								n/o
								3.7 h
								n/o
								6.0 h
								1.2 h

n/o = not observed; - = no sample. Bradycardia is defined as a drop of 25% from predosing heart rate (30-minute average).

Cholinesterase activities (μmol acetylthiocholine hydrolysed/min/(ml blood) or per 100mg brain tissue) in selected tissues following VX (0.74 mg/kg p.c.) and in naïve weight-matched control animals from a previously published study (Mumford et al 2008b).

Table 2

Treatment group	Tissue						
	Striatum	Hippocampus	Cortex	Midbrain	Pons	Cerebellum	Erythrocytes Plasma
Naïve controls; n=6	2.35 $\pm$ 0.23	0.65 $\pm$ 0.03	0.60 $\pm$ 0.04	0.84 $\pm$ 0.07	0.91 $\pm$ 0.14	1.42 $\pm$ 0.08	0.70 $\pm$ 0.13 1.26 $\pm$ 0.21
VX + saline (2h)	1.43 (60.8)	0.37 (56.9)	0.38 (63.3)	0.49 (58.3)	0.44 (48.4)	0.86 (60.6)	- 0.03 (2.4)
4.17h, 6.67h; n = 2	1.59 (67.6)	0.62 (95.4)	0.47 (78.3)	0.75 (89.3)	0.69 (75.8)	0.98 (69)	0.16 (22.8) 0.03 (2.4)
VX + Protexia (2h)	2.21 $\pm$ 0.45	0.52 $\pm$ 0.12	0.53 $\pm$ 0.07	0.71 $\pm$ 0.09	0.73 $\pm$ 0.15	1.14 $\pm$ 0.19	1.15 $\pm$ 0.35 9.93 $\pm$ 7.93
7 days; n=8	(94) ns	(80) †	(88.3) ns	(84.5) ‡	(80.2) †	(80.3) ‡	(164.3) † (788.1) †

Figures in parentheses show percent cholinesterase activity of control animals. Animals received Protexia or saline 2h following VX. Due to overnight deaths, the saline group comprised a limited number of animals (n=2) from which samples were obtained at the times indicated; - = no sample. Samples from Protexia-treated animals were obtained at 7 days. Data shown are mean $\pm$ SD. Significance for comparisons of each tissue to naïve control group ns=not significant. Unpaired two-tailed t-test.

† p<0.05,

‡ p<0.01,