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RESPIRATORY AND CIRCULATORY EFFECTS OF SAXITOXIN IN THE CEREBROSPINAL FLUID

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1 In cats anaesthetized with pentobarbitone, saxitoxin and, on a few occasions, tetrodotoxin were injected into a lateral cerebral ventricle or into the subarachnoid space of the lower brain stem. Observations were made on frequency and tidal volume of breathing, on CO_2 responsiveness and on electrical responsiveness of the respiratory centre. Effects on the blood pressure were observed simultaneously.

2 A single large dose of toxin, e.g., 250 ng, produced within minutes apneustic breathing and a rise in blood pressure which were converted rapidly to respiratory failure and hypotension. In contrast, repeated small doses, e.g., 25 ng, yielded only progressive slowing of the respiration together with circulatory hypotension. Bulbar depression was produced as effectively by subarachnoid injection as by intraventricular injection of the toxins. Onset of action was detectable within seconds.

3 Slowing of the respiration occurred independently of change in tidal volume and whether or not the vagus nerves were cut. The reduction in breathing frequency is attributed to direct toxin-induced depression of the central respiratory oscillator.

4 Steady-state measurements of tidal volume at controlled levels of alveolar CO_2 pressure in intermediate stages of respiratory depression showed that the toxins produced an increase in CO_2 stimulation threshold as well as a reduction in gain of CO_2 responsiveness, whether or not the vagus nerves were cut. Carotid arterial chemoreceptor reactivity to O_2 was demonstrable when central sensitivity to CO_2 was depressed. These effects are attributed to a direct influence of the toxins upon the brainstem CO_2-tidal volume controller.

5 Responsiveness of the medullary inspiratory centre to electrical stimulation persisted after the failure of spontaneous breathing was caused by the toxins. Conversely, restitution of electrical responsiveness preceded the reappearance of spontaneous respiratory activity in the recovery phase of toxic depression. Circulatory effects paralleled the changes in respiratory behaviour.

6 On the basis of the relatively prompt and discrete alterations in the central respiratory and circulatory control mechanisms produced by saxitoxin and tetrodotoxin placed in the cerebrospinal fluid, it is concluded that the agents rapidly penetrated to deep target loci in the lower brain stem.

Introduction

The respiratory failure that occurs in saxitoxin (STX) poisoning, from the ingestion of contaminated shellfish, is generally attributed to neuromuscular paralysis. The same is true for tetrodotoxin (TTX), the puffer fish poison (Evans, 1972). Indeed, despite their chemical dissimilarity, the two substances are thought to behave pharmacologically in an identical fashion. We have reported on the remarkable effectiveness of TTX in altering respiratory function after intracerebroventricular injection in the cat (Borison, McCarthy, Clark & Radhakrishnan, 1963). Jaggard & Evans (1975) described the respiratory depressant effects of STX injected intraventricularly in rabbits with the notable observation that apneustic breathing was not produced in the rabbit as was found in the cat.

The present study was undertaken in cats to define further the actions of STX placed in the cerebrospinal fluid (CSF) on the central control components of the respiration where actions on the neuromuscular effector system are excluded from taking part. Concurrent observations on the arterial blood pressure afforded us the opportunity to draw conclusions applicable to central vasomotor control. TTX was employed to a limited extent for comparison with STX.

These experiments cannot resolve the standing controversy on whether STX and TTX enter the brain from the blood. However, we are concerned with the movement of the toxins from the CSF into the brain, and with changes in the character of the breathing which may bear upon the nature of the respiratory failure that occurs in cases of seafood poisoning from STX and TTX.

Methods

Experiments were performed on 12 cats anaesthetized with pentobarbitone sodium (40 mg/kg injected intraperitoneally). Small supplemental doses of the anaesthetic were administered intravenously as required during the course of long experiments. The trachea was cannulated low in the neck, a catheter was placed in a suitable vein for systemic administration of drugs and the blood pressure was obtained from the femoral or carotid artery depending on accessibility according to physiological recording conditions. Respiratory excursions were recorded by any of four techniques, namely, a whole body plethysmograph, a half-body plethysmograph from which the head and shoulders protruded, an elastic body sleeve enveloping a pneumatic cuff, and a pneumotachograph attached to the tracheal cannula. Selection of a particular technique for recording the respiration was dictated in good measure by the use in certain experiments of a cranial stereotoxic instrument for holding stimulation electrodes and injection cannulae.

Respiratory gas tensions were routinely monitored through a sampling catheter in the trachea by means of a Beckman infra-red CO_2 analyzer and a Westinghouse O_2 analyzer. The pertinent physiological variables were recorded continously on a Brush 6-channel rectilinear oscillograph. Body temperature was controlled within normal limits.

Injections of STX and TTX into the cerebrospinal fluid were made through stereotaxically placed cannulae in a lateral cerebral ventricle (McCarthy & Borison, 1966) or in the subarachnoid spaces on both sides of the medulla oblongata at the level of the lateral apertures. The cannulation sites were validated by injection of dye at the end of the experiments.

Electrical stimulation of the medial reticular formation in the medulla oblongata was accomplished with a bipolar nichrome wire electrode, less than 1 mm in diameter, positioned stereotaxically below the level of the acoustic striae. The electrical stimulus consisted of biphasic pulses of one ms duration at a frequency of 50 per s delivered by a Grass stimulator. The stimulus strength was adjusted to yield a near-maximal sustained inspiratory response for intervals of up to 10 seconds. Reproducible changes in blood pressure were elicited concurrently with the evoked inspiratory response. The stimulus current was monitored by means of an in-series oscilloscope to detect changes in flow path resistance over long testing periods; no changes of consequence were observed.

Central respiratory responsiveness to CO_2 was assessed by measuring steady-state tidal volume amplitudes at selected alveolar pressures of CO_2 (Pa_{CO_2}). Hypercapnic levels were achieved by controlled administration of CO_2 in the inspired gas. Hypocapnic levels were achieved by means of artificial hyperventilation which was suddenly discontinued to obtain the spontaneous tidal volume amplitude corresponding to the reduced $Paco_2$. O_2 levels were controlled concomitantly through a gas mixing manifold.

Saxitoxin was kindly supplied by Dr E.J. Shantz; it was dissolved in 0.001 N HCl to a stock concentration of 500 μg/ml. Tetrodotoxin was obtained through the courtesy of the Sankyo Drug Co., Tokyo. All doses were diluted from a stock solution of 10 μg/ml in 0.9% w/v NaCl solution (saline). The stock solutions were stored at a temperature below freezing.

Results

Influence of saxitoxin and tetrodotoxin on respiratory pattern and blood pressure

STX was injected into a lateral cerebral ventricle in 9 cats at concentrations of 0.1 μg/ml and 1.0 μg/ml in volumes from 0.1 to 0.25 ml delivered. The lowest concentration often required repeated administration in order to produce a noteworthy effect despite the large volume injected. However, prompt changes in breathing and in blood pressure were elicited with minimally effective doses as shown in Figure 1. In this case, the response to a second injection of 25 ng in 0.25 ml consisted of an immediate brief tachypnoea with consequent reduction of $Paco_2$ followed by respiratory slowing and irregularity that resulted in elevation of $Paco_2$. The blood pressure is seen to have increased within a minute from the start of injection even before $Paco_2$ began to rise. Still slow but improved breathing developed within the next 15 minutes. The initial tachypnoeic response is consistent with the hypothermia evoked by intracerebroventricular STX and TTX in unanaesthetized cats (Borison *et al.*, 1963; Clark & Lipton, 1974). Elevation in blood pressure has been observed previously to occur in response to TTX as well as to procaine (Borison *et al.*, 1963; Borison, Haranath & McCarthy, 1972). It provides a plausible explanation for the pulmonary oedema that was precipitated by TTX in awake cats, reported by us in an earlier investigation (Borison *et al.*, 1963).

The progression of events following injection of a large dose of STX, namely 250 ng in 0.25 ml into a lateral ventricle, is shown in Figure 2. An attempt was made here to maintain isocapnic conditions by starting at an elevated $Paco_2$ level and reducing the CO_2 tension in the inspired gas as respiratory depression developed. At 3 min after the injection, the respiratory rate was already reduced by approximately 50% while tidal volume (V_T) was only modestly decreased and mean blood pressure had risen to 200 mmHg. By 7 min, intermittent apneustic breathing had appeared with V_T persisting at an undiminished amplitude; a disturbance in cardiac rhythm was then evident. By 14 min, apneustic breathing was fully developed and it was no longer possible to control $Paco_2$. Thereafter, over the course of 1 h, V_T faded progressively to the

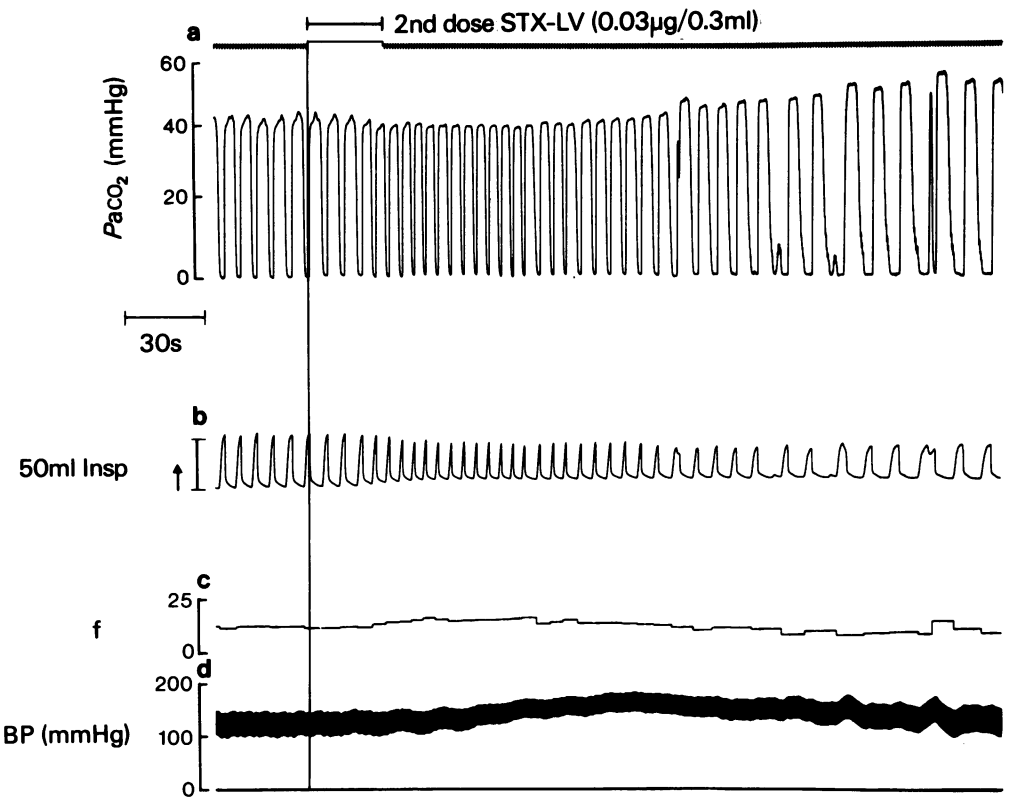

Figure 1 Effects on respiration and blood pressure of a small dose of saxitoxin (STX) injected into a lateral ventricle (LV); the total amount administered was 25 ng in 0.25 ml after subtracting cannula deadspace. (a) Endotracheal CO_2 tension; peak values represent alveolar partial pressure of CO_2 (P_{aCO_2}). (b) Respiratory excursions were recorded plethysmographically, inspiration upwards. (c) Frequency of breathing (f) was recorded by a tachometer operating off the plethysmograph signal. (d) Arterial blood pressure (BP).

point where artificial ventilation became mandatory; blood pressure decreased concomitantly. Spontaneous breathing was first manifested again at about 5 h after the injection of STX.

STX (in three cats) and TTX (in two cats) were injected bilaterally into the subarachnoid space of the posterior fossa at the lateral apertures of the fourth ventricle. Greater variability in effects of the toxins was encountered with this technique than with intraventricular injection, possibly because of the small volume of solution injected, i.e., 0.05 ml per side. In general, true apneustic respiration was more difficult to achieve with subarachnoid injection; breathing more often slowed to a stop while blood pressure drifted downward as contrasted with the hypertensive effect obtained after intraventricular injection. Nevertheless, paramedullary subarachnoid injection proved to be a powerful means of producing respiratory failure. For example, in one cat two injections of 50 ng STX given unilaterally resulted in respiratory ataxia after which a single contralateral injection of 20 ng promptly converted the breathing into an apneustic pattern.

No difference was evident in the nature of the effects produced by STX and TTX. Indeed, in one unanaesthetized cat, STX was injected intraventricularly in small spaced doses that evoked a generalized behavioural syndrome indistinguishable from that seen with TTX. Breathing was not compromised until the animal was anaesthetized with pentobarbitone at which time respiratory failure ensued. Thus, interaction with general anaesthesia may well be decisive for the nature of the respiratory depressant effect produced by the marine toxins acting centrally.

Influence of the toxins on respiratory frequency vs. tidal volume

Respiratory frequency (f) normally varies directly in proportion to the tidal volume (V_T) of breathing but independently of changes in concentration of CO_2 and O_2 in the blood (Rosenstein, McCarthy & Borison,

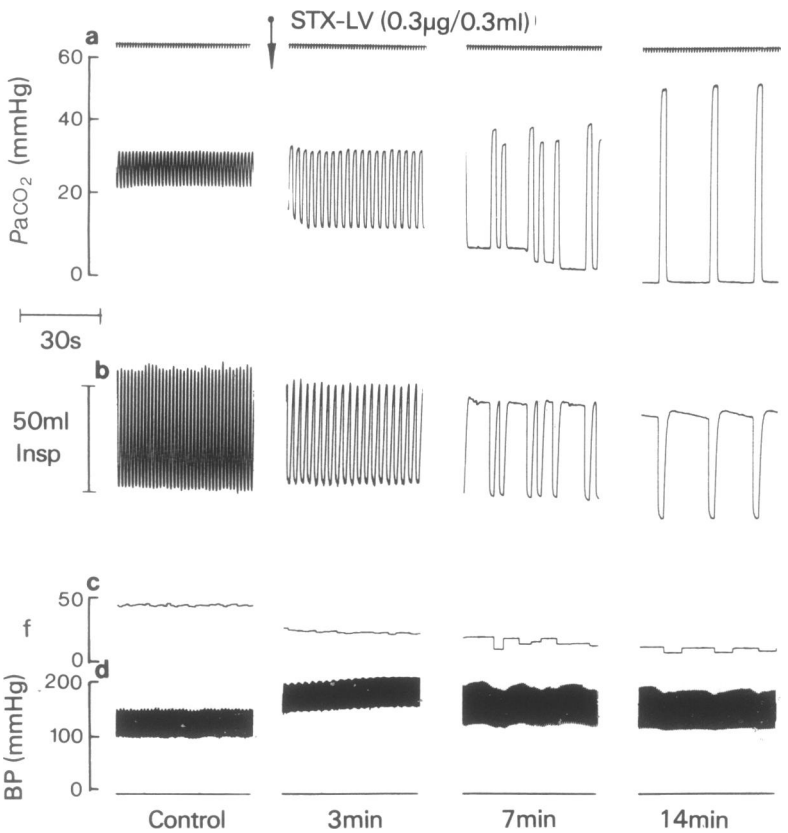

Figure 2 Effects on respiration and blood pressure of a large dose of saxitoxin (STX) injected into a lateral ventricle (LV); the total amount administered was 250 ng in 0.25 ml after subtracting cannula deadspace. Polygraph tracings are presented as in Figure 1. The cat was initially stabilized at a P_aCO_2 level of 32 mmHg through inhalation of CO_2 in air at 22 mmHg. Note the reduction in administered CO_2 to compensate for reduced ventilation, and accumulation of endogenous CO_2 resulting from the action of saxitoxin. After 7 min, CO_2 was completely withdrawn from the inspired gas.

1973). Vagotomy consistently abolishes the dependence of f upon V_T, resulting in a fixed breathing frequency at all depths of breathing.

Figure 3 shows the sequential effect on the f vs. V_T relationship of a small dose of STX injected twice into the lateral cerebral ventricle of a non-vagotomized cat. The injections of STX (25 ng per dose) serially reduced breathing frequency and the $\Delta f/\Delta V_T$ slope. Vagotomy then produced no further reduction in f. This notwithstanding, the effect of one more injection of STX (not shown in the figure) was an additional slowing of the respiration leading finally to failure. Thus, the decelerating effect of STX on respiratory frequency and the apparent uncoupling of f from V_T does not depend on the status of the vagus nerve. Rather, STX acts directly on the respiratory oscillator to reduce its driving frequency below the feedback operating level of the vagus nerve.

In the experiment shown in Figure 4, vagotomy was performed at the outset thereby causing the resting respiratory rate to fall from 24 to 12 breaths per minute. Nevertheless, the frequency was then further reduced following each successive injection of STX, which serves to substantiate the direct central influence of the toxin on the frequency controller.

Influence of the toxins on CO_2 responsiveness

STX and TTX was administered to each of five cats in partial depressant doses to yield fairly stable subparalytic stages in the course of their cumulative influence on the central CO_2–V_T controller. No distinction is made between intracerebroventricular and subarachnoid injections or between the responses to STX and TTX.

Figure 4 illustrates the effect of successive doses on

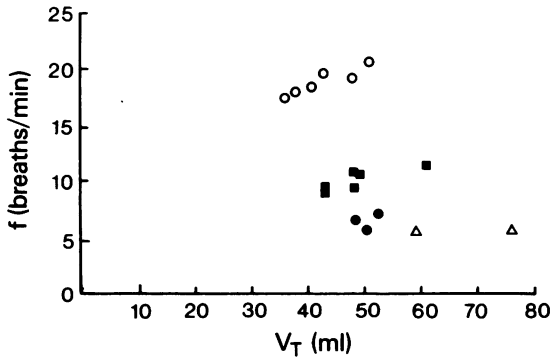

Figure 3 Effect of repeated small doses of saxitoxin injected into the cerebrospinal fluid on the relationship of respiratory frequency (breaths per min, f) to tidal volume (V_T). Measurements were made during steady-state conditions at various controlled levels of P_{aCO_2} given to produce changes in V_T. Note the failure of bilateral vagotomy further to reduce f after the second dose of saxitoxin. (○) Control; (■) saxitoxin 30ng/0.3ml; (●) + saxitoxin 30ng/0.3ml; (▲) vagotomy.

the V_T vs. log P_{aCO_2} relationship in a cat that was initially vagotomized to eliminate pulmonary proprioceptive feedback. Except for an increase in CO_2–V_T response gain produced by the vagotomy in this case, the results of drug administration were sufficiently alike in the entire group of cats to permit the following generalizations. Repeated injections of toxin

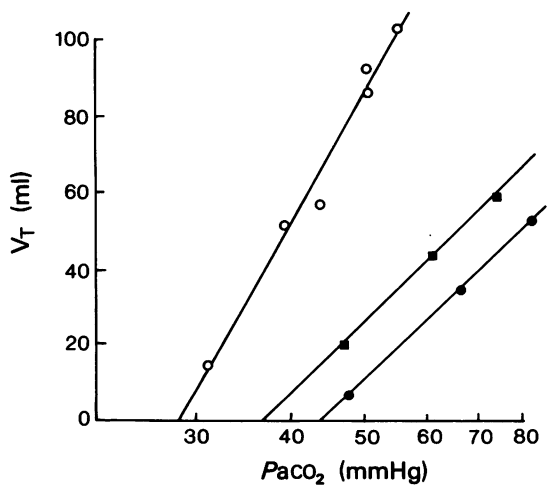

Figure 4 Effect in a vagotomized cat of repeated small doses of saxitoxin injected into the cerebrospinal fluid on the relationship of tidal volume (V_T) to alveolar CO_2 tension (P_{aCO_2}), plotted semilogarithmically. Note the shift to the right and reduction in slope produced by the saxitoxin. (○) Control; (■) saxitoxin 30ng/0.3ml; (●) + saxitoxin 30ng/0.3ml.

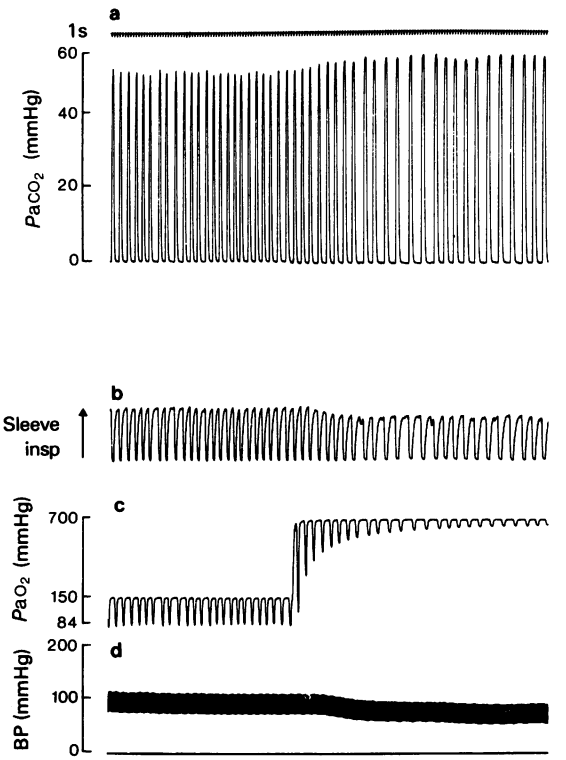

Figure 5 Influence of sudden oxygen inhalation on the respiration in an intermediate stage of depression produced by saxitoxin. Respiratory movement was recorded by means of an elastic body sleeve enveloping a pneumatic cuff. The delivery of O_2 to the airway is indicated in the tracing of P_{aO_2} (c) by the step increase of peak values from 150 to 700 mmHg. Alveolar partial pressure of O_2 corresponds to the troughs in the recording. Note the prompt reduction in amplitude and rate of breathing (b), the rise in P_{aCO_2} (a) and the fall in blood pressure (d) resulting from the interruption of peripheral vascular chemoreceptor discharge in response to the inhalation of oxygen.

resulted in a widening shift to the right and a decreasing slope of the V_T/log P_{aCO_2} function until a flat response line was achieved at the last stage before respiratory failure.

The functional status of peripheral chemoreceptor feedback, particularly from the carotid body, was evaluated in several experiments by suddenly raising and lowering the inspired O_2 tension. Appropriate ventilatory responses of diminution and enhancement, respectively, in the tidal volume were obtained in the late as well as early stages of STX influence upon spontaneous breathing. The ventilatory effect of a step inhalation of 100% O_2 during an intermediate stage of STX depressant action is shown in Figure 5. Thus it is evident that the major central effects of the toxins on

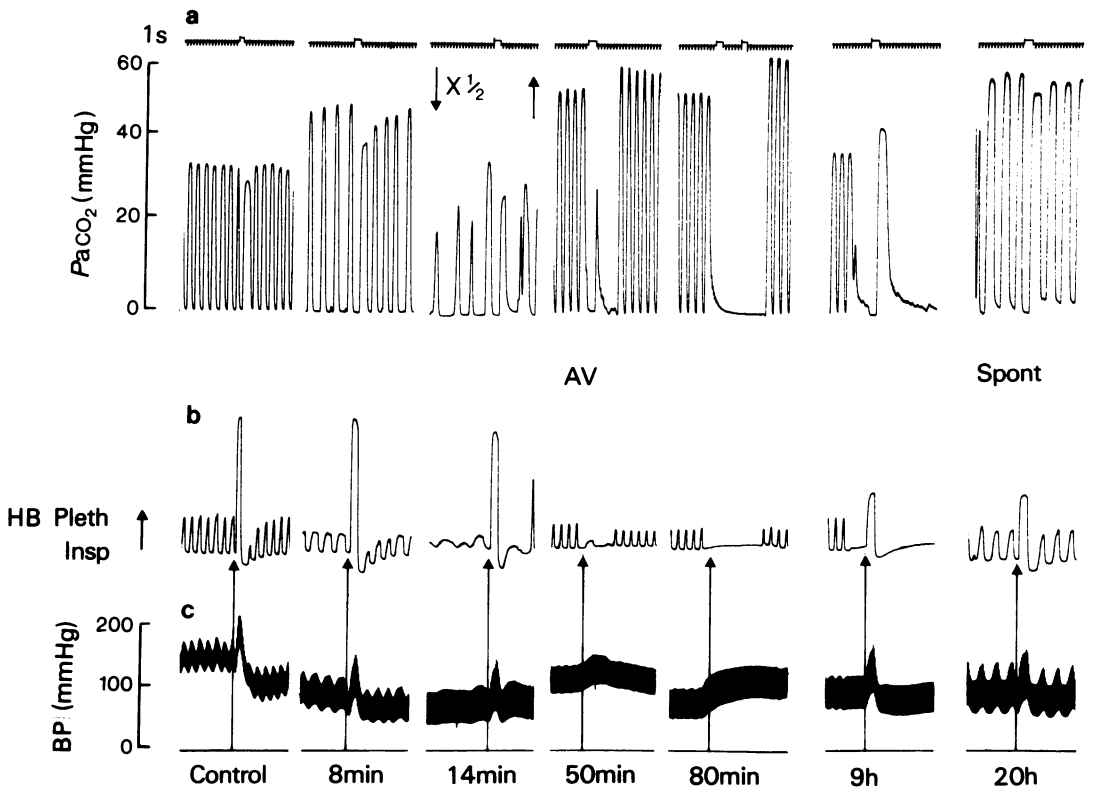

Figure 6 Effects of saxitoxin on spontaneous breathing (b) and blood pressure (c) compared with effects on the responses to electrical stimulation of the medullary reticular formation. Respiratory movements were recorded by means of a half-body plethysmograph (HB Pleth) from which the head and shoulders protruded to permit the use of a cranial stereotaxic electrode-holding device. Artificial ventilation (AV) was started immediately after the electrical stimulation test, at 14 min after the administration of saxitoxin. (a) Amplification of the CO_2 recording was reduced by one-half during the final stage of respiratory failure. Recovery of spontaneous ventilation (Spont) was evident at 20 h after injection of the toxin. Inspiratory electrical responsiveness, indicated by the upright arrows from the baseline, failed later and recovered earlier than did spontaneous breathing. Cardiovascular pressor responses to electrical stimulation showed parallel effects of the toxin. Methoxamine was administered intermittently to raise the blood pressure following the collapse of respiratory and circulatory homeostasis.

respiratory pattern and CO_2 responsiveness are not attributable to the interruption of afferent impulse traffic which could possibly result from a local anaesthetic action upon nerve rootlets traversing the subarachnoid space.

Influence of the toxins on the response to electrical stimulation of the respiratory centre

Amplitude of the response. Electrical stimulation of the medial reticular formation in the lower brain stem was carried out in seven cats of which five were given STX and the others received TTX. An electrode position was selected that yielded a brisk, deep, sustained inspiratory effort with a quick off-response and rapid recovery of spontaneous function (see Figures 6 and 8). Wide opening of the mouth and protrusion of the tongue occurred regularly as part of the integrated inspiratory event, accompanied by little or no extraneous body movement. The blood pressure response to electrical stimulation bore no constant relationship to the respiratory response, although the direction of change in blood pressure elicited from a particular medullary locus remained consistent throughout the stimulation session. A suitable stereotaxic placement of the electrode, once established, was fixed for the remainder of the experiment since essentially no deterioration in responsiveness to submaximal stimulation was evident over many trials under control conditions.

Figure 6 illustrates the dissociation of spontaneous and evoked respiratory behaviour produced by 250 ng

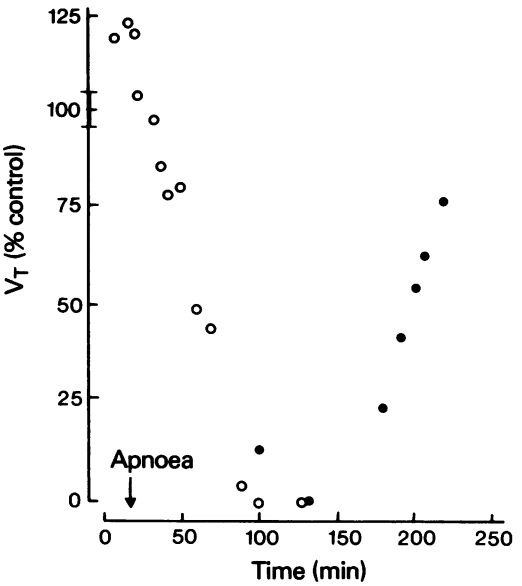

Figure 7 Time course of the effect of tetrodotoxin on the tidal volume (V_T) response to electrical stimulation of the inspiratory centre. Change in responsiveness is expressed as fractional % of the control (pre-drug) amplitude. The bracket on the ordinate scale signifies the standard deviation of responses to a 3V stimulus (50 pulses per s; pulse duration of one ms) before drug injection. Failure of spontaneous breathing (apnoea) occurred at 18 min after injection of tetrodotoxin into the cerebrospinal fluid when electrical responsiveness was 25% greater than the pre-drug activity. Thereafter, the evoked response declined linearly to zero at 100 min at which time the strength of stimulation was increased to 6 volts. At 130 min, the 6V stimulus also became ineffective. Recovery then followed continuously to near completion over the next 100 minutes. Artificial ventilation was interrupted for electrical stimulation test purposes. (O) 3V (64μA); (●) 6V.

of STX injected into a lateral cerebral ventricle. At 8 min after the injection, breathing rate was dampened remarkably, resulting in a considerable elevation in $Paco_2$. Nevertheless, the electrically-evoked inspiratory response was practically unaffected. By 14 min, spontaneous breathing was all but abolished, yet the response to electrical stimulation was only slightly affected. Artificial ventilation was then instituted and continued to be needed until the next morning. The blood pressure too required support and was bolstered by judicious injections of methoxamine over the first few hours. The respirator was turned off periodically for testing the effect of electrical stimulation which became totally ineffective by 80 min after injection. At 9 h, partial recovery of central electrical responsiveness had become evident while spontaneous respiration only reappeared after 20 h following the injection. Opening of the mouth and movement of the tongue persisted when inspiratory movement could no longer be evoked by electrical stimulation of the medulla. This means that the cranial nerves involved were not disabled by a non-specific local anaesthetic action of STX. Furthermore, the blood pressure response to electrical stimulation was influenced by STX in a temporal order that paralleled the changes in the evoked respiratory response.

Figure 7 shows the time course of change in amplitude of the evoked inspiratory effort following the injection of TTX (1.0 μg total) bilaterally into the subarachnoid space. Spontaneous breathing ceased at 18 min, at a time when the respiratory response was enhanced. At 100 min, the original stimulus strength of 3 V was no longer effective and so the voltage was doubled. This too became ineffective at 130 minutes. Thereafter during the next hour the response proceeded rapidly to reverse towards normal, still with no display of spontaneous breathing activity.

Onset of the evoked inspiratory response. An additional combination of effects that emerged in the course of central respiratory depression produced by the toxins was a prolongation in latency, as well as in rise time, of the electrically-induced inspiratory response. This is shown in Figure 8 in which the same experiment is represented as in Figure 7. The chart speed of the recording had been increased to permit the estimation of response latency to the nearest 10 milliseconds. At 10 min after the injection of TTX when spontaneous breathing was grossly depressed but the response amplitude was actually enhanced, no change was evident in the latency or in the rise time of the evoked inspiration. The first sign of central transmission blockade, namely, a small though unmistakeable slowing in the rate of rise, appeared 20 min after the injection, coinciding approximately with the need for artificial ventilation; latency, however, was still unaffected. By 100 min, both latency and rise time were prolonged despite the increment in stimulus strength from 3 to 6 volts. The next panel shows that the evoked response continued to be further delayed in onset even though amplitude recovery had begun. With further improvement, both latency and rise time shortened together as the effect of the toxin wore off.

Discussion

We have attempted in this study to examine actions of STX and TTX on the control components of the respiration and circulation when the substances are placed in contact with the brain. The results indicate that the toxins affected structures in the brain stem too far removed from the surface to be accessible solely by diffusion from the CSF.

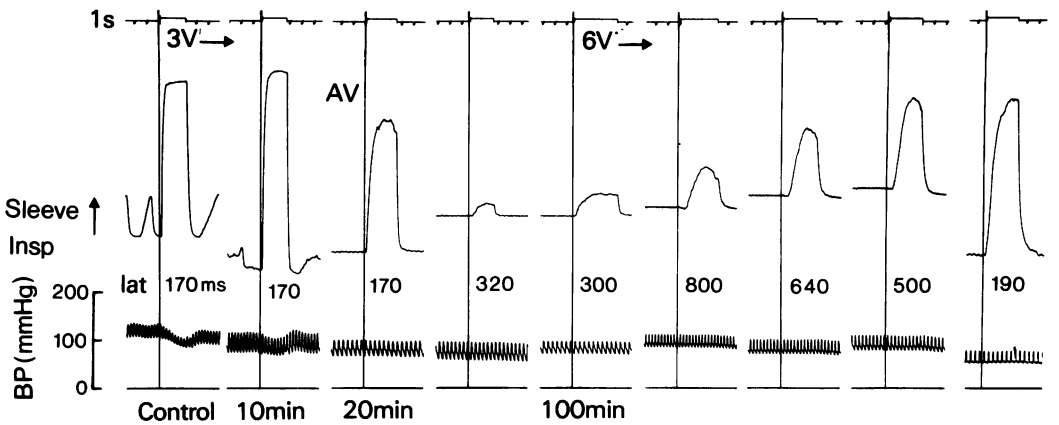

Figure 8 Effect of tetrodotoxin administered into the cerebrospinal fluid on the inspiratory response to electrical stimulation of the medullary reticular formation. This recording is from the same experiment as Figure 7. Respiratory movement was recorded by means of an elastic body sleeve enveloping a pneumatic cuff. The polygraph paper speed permitted micrometer estimation of response onset latency to the nearest 10 milliseconds. The latency measurement (lat) is given below every evoked response. Artificial ventilation was started at 18 min and was interrupted briefly for each stimulation test. The stimulus strength was increased from 3 to 6V at 100 min after the drug injection. The latency of response onset continued to be further prolonged even after improvement in the response amplitude had become manifest. Methoxamine was administered intermittently to elevate the blood pressure.

Dissociation of respiratory control components

The central controlling apparatus of breathing consists of two major divisions, namely that concerned with the control of frequency (f) and the other with tidal volume (V_T). The available evidence overwhelmingly supports the contention that the blood gases have no direct influence on the control of respiratory periodicity by the hindbrain, but that they affect only the depth of breathing through their respective chemosensory inputs (Flórez & Borison, 1967; Borison & McCarthy, 1973; Rosenstein, McCarthy & Borison, 1974). Nevertheless, indirect changes in f normally accompany CO_2- and O_2-induced changes in V_T through the Hering-Breuer vagal reflex mechanism. Thus, a drug-provoked disturbance of periodicity, whether in frequency or in pattern, particularly without an associated change in tidal volume and/or following vagotomy, must be attributed to a functional alteration in the oscillatory control mechanism occurring separately from any influence upon the amplitude control mechanism. It is generally agreed that hypoxia stimulates breathing solely through activation of peripheral vascular chemoreceptors, i.e., the carotid body, whereas the major feedback influence of CO_2 on V_T is mediated by central chemoreceptors, possibly located on the ventrolateral surface of the lower brain stem. Accordingly, a drug-induced change in tidal volume under controlled blood gas conditions, particularly without an associated effect upon peripheral chemoreceptor input, must be attributed to a functional alteration in the CO_2-V_T control mechanism occurring separately from any influence upon the central oscillatory apparatus. Lastly, it is possible to evaluate a separate drug influence on the final integrator of inspiratory movement by means of electrical stimulation of the medullary reticular formation.

The results of the present experiments demonstrate at least three independent actions of the toxins on the respiratory centre. The earliest and most prominent effect took the form of changes in frequency and pattern of breathing assignable to an action on the respiratory oscillator and the associated elements that control periodicity. The second action was manifested upon the tidal volume controller by shifts in position and slope of the CO_2-V_T response line that were obtained in intermediate stages of central depression before the attainment of complete block of periodicity. Additionally, electrical responsiveness of the medial reticular formation outlasted the inactivation of both frequency control and CO_2-tidal volume control, and so a depressant influence on the inspiratory output integrator represents the third central action of the toxins. The relative invulnerability to the toxins of the final common pathway from the respiratory centre to the lower motor neurone is supported by the simple observation that gasping occurred terminally following the failure of spontaneous breathing.

We have no reason to believe that local anaesthesia of nerve roots which traverse the subarachnoid space contributed to the observed effects. Firstly, toxin-induced alterations in respiratory pattern and in CO_2 responsiveness were shown to be independent of

pulmonary and peripheral chemoreceptor innervation. Secondly, and more importantly, coordinated cranial inspiratory auxiliary movements could be elicited by central electrical stimulation after spontaneous respiratory activity had ceased.

Jaggard & Evans (1975) emphasized an apparent species difference in the effect of the toxins in rabbits and cats, pointing out that apneustic breathing was never observed by them in the rabbit. However, they did report a prolongation of the inspiratory phase along with the slowing of breathing which suggests a tendency towards apneustic respiration. Considering the multiple depressant actions exercised by the toxins, the complex cumulative effects of repeated doses, and the superimposed depressant influence of anaesthesia, the differences in responses to the toxins reported for cats and rabbits would not appear to represent a real distinction between species. Indeed, in the present work on cats, when repeated small doses were given to obtain intermediate levels of respiratory depression required for steady-state measurements of CO_2 responsiveness, the breathing slowed in a number of instances without passing through a frank apneustic phase.

Access of the toxins to their sites of action

No qualitative differentiation could be made from the present experiments between the respiratory effects obtained with the intraventricular and subarachnoid injections. Prompt changes in breathing were elicited by the toxins in either case. This indicates that the involved neural targets were reached even when the substances did not come in contact with the ventricular lining.

The same questions that are invariably linked to the effects of substances placed into the cerebrospinal fluid are how fast and how far the agents penetrate into the brain and spinal cord. The toxin-induced conversion of eupnoea to apneustic breathing is presumed to result from selective depression of the pneumotaxic centre located in the floor of the fourth ventricle (Tang, 1953), but separated by several millimeters from the pial surface of the hindbrain (Berman, 1968). Other structures concerned in the generation and modulation of the respiratory waveform are situated deep in the medulla. On the other hand, the ventrolateral surface of the brain stem has increasingly been receiving attention as a region of pharmacological sensitivity both for respiratory and cardiovascular responses (see Feldberg, 1976). However, the pial surface is densely vascularized with the stem vessels of branches that penetrate as far as the dorsal ependymal surface of the pons and medulla (Crosby, Humphrey & Lauer, 1962). The pia-arachnoid membrane itself consists of a spongy meshwork of connective tissue fibres in which the blood vessels are embedded, yet responses to local drug application are elicitable within seconds. We have shown previously that procaine, like TTX and STX produces apneustic breathing after only 5 s of drug delivery to the brain surfaces by means of rapid ventriculocisternal perfusion (Borison *et al.*, 1972). There are no known mechanisms by which such a precipitous action could be effected by passive diffusion to appropriate responding elements.

Starting with the simplest geometric diffusion model (Riggs, 1963) and assuming a diffusion coefficient for TTX equal to that of sucrose, i.e. 3×10^{-6} cm^2/s (Colquhoun, Henderson & Ritchie, 1972; Patlak & Fenstermacher, 1975) we calculated that 50% fractional equilibrium is reached at 0.05 mm in 10 s, and that 4 h would be required to reach the 50% concentration level at 2 mm from the brain surface. Hence, we are faced with the quandary that neither passive nor active processes have yet been defined that provide a suitable explanation for the nature and timing of the observed pharmacological effects. Nevertheless, it seems unreasonable to attribute a broadly diversified spectrum of drug-induced responses to selective sites of chemosensitivity on the pial surface in the absence of convincing neurohistological evidence for the existence of superficial receptive elements, and in the presence of a potential vascular transport system into deep structures of the brain. It is germane that the toxins as well as local anaesthetics do not directly suppress receptor sensitivity to a specific stimulus since the generator potential is not affected by blockade of sodium activation gates (Loewenstein, Terzuolo & Washizu, 1963). Thus, nerve fibres connecting the putative surface receptors to interior structures would have to be readily accessible from the cerebrospinal fluid if the diffusion postulate is to remain credible. Additionally, the effects of the toxins on the latency and rise time of the inspiratory response to medullary electrical stimulation point to a direct blocking influence on neural recruitment at the output step of respiratory integration. This requires a generalized action on interneuronal transmission in the reticular formation, not involving a chemoreceptive input.

We reported previously that TTX in doses of 1.0 and 2.0 μg was ineffective in producing respiratory arrest when given by intracisternal injection (Borison *et al.*, 1963). This is in contrast with the results reported by Li (1963) and is in apparent conflict with our present findings after subarachnoid injection. However, the discrepancies can be reconciled by taking into account possible differences in distribution of the injectates. Bilateral paramedullary injection as performed here closely simulates the egress of ventricular fluid through the foramina of Luschka; the cat has no foramen of Magendie. An intracisternal injection on the other hand could well be distributed asymmetrically in the subarachnoid CSF and, depending on the volume injected, could pass mainly in a caudal direction thereby missing to a large extent the ventral surface of the brain stem.

Blood pressure and other functional considerations

We have observed a sizeable increase in blood pressure as a regular concomitant to the elicitation of apneustic breathing by TTX (Borison *et al.*, 1963), by procaine (Borison *et al.*, 1972) and by STX in the present study. The hypertensive effect was not seen after repeated small doses that resulted in slowing without apneustic breathholding. In such instances the blood pressure showed a continuing decline along with the respiratory depression. It is evident from these results that an overall disinhibition of medullary activity occurred as an early response to a suitable dose of toxin acting at the pontine level. This mode of action provides an explanation also for the enhancement in the inspiratory response to electrical stimulation that was occasionally observed initially in the course of the drug effect before the development of apnoea (see Figures 7 and 8).

The demonstration by Clark & Lipton (1974) that the hypothermic response to STX and TTX represents a lowering of setpoint rather than a paralysis of thermoregulation suggests by analogy that the shift in CO_2 responsiveness includes an upward resetting of the CO_2 regulatory setpoint. We did in fact observe in one experiment a parallel shift of the V_T/CO_2 response function before a reduction in slope resulted from injection of toxin. Parallel shifts in the CO_2-ventilation response line are produced normally by physiological shifts in setpoint as, for example, in the transition between waking and sleep states (Reed & Kellogg, 1958). On the other hand, neuronal depressants such as general anaesthetics can reduce the slope without shifting the position of the CO_2-V_T line (Flórez & Borison, 1969). Hence, the toxins may influence CO_2 responsiveness in two ways, namely by an upward shift in setpoint and by a reduction in gain.

Does central respiratory depression occur in systemic intoxication?

Kellaway (1935) concluded that 'mussel poison' acts centrally despite the fact that phrenic nerve discharges persisted after respiratory paralysis had resulted from intravenous injection of the toxin in rabbits. He reasoned correctly that the respiratory slowing which first developed in his 'unbuffered' (peripherally denervated) animals could not be explained through a peripheral depressant action. Prinzmetal, Sommer & Leake (1932) even earlier, described a characteristic slowing of the respiration that resulted cumulatively from repeated intravenous injections of mussel poison in dogs and rabbits. The effect was not modified by section of the vagus nerves. Thus, a change in character of breathing is surely a more revealing indication of central depression than is respiratory arrest. Moreover, we have observed active gasping after central respiratory failure in the present experiments. This finding complicates the often cited conclusion that no central depression occurs after systemic administration of the toxins because phrenic discharges have been recorded in the presence of respiratory paralysis. Such phrenic activity could in fact represent the last vestige of central respiratory function after severe depression.

One cannot help but be impressed by the extraordinary toxic potency of STX and TTX when they are placed in direct contact with the brain. It is therefore most surprising that a single line of positive evidence derived from phrenic nerve potentials is used to exclude the possibility that the toxins do not penetrate the brain from the blood. Data from cross-circulation experiments reported by Kao, Suzuki, Kleinhaus & Siegman (1967) constitute negative results which leave room for doubt. Curiously, Wang, Huang, Kahn & Wang (1968) concluded from similar experiments presented in a preliminary report that TTX stimulated respiration centrally but that it depressed the vasomotor centre. It is at best difficult to reconcile the many indications of central action observed both clinically and experimentally with the unproved contention that the blood-brain barrier is not permeable to the toxins. Neurological effects of systemic intoxication include vomiting, paresthesias, cerebellar signs, floating sensations, impairment of perception and speech aphasia (Yudkin, 1944; Seven, 1958). A more direct experimental proof of central influence is the demonstration that TTX depressed medullary reticulospinal inhibition of spinal reflex activity (Frank & Ohta, 1972). In our opinion, the balance of opposing arguments favours the contention that central nervous system depression contributes to the syndrome of shellfish and puffer fish poisoning.

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