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# CENTRAL RESPIRATORY AND CIRCULATORY EFFECTS OF *Gymnodinium breve* TOXIN IN ANAESTHETIZED CATS

HERBERT L. BORISON, SYDNEY ELLIS[1] & LAWRENCE E. McCARTHY

Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, New Hampshire 03755, U.S.A.

**1** In cats anaesthetized with pentobarbitone, observations were made on respiration, spontaneous and evoked diaphragmatic electromyograms, blood pressure, heart rate, indirectly-induced contractions of the anterior tibialis muscle and nictitating membrane, and electrical excitability of the inspiratory centre in the medulla oblongata.

**2** *Gymnodinium breve* toxin (GBTX) was administered intravenously, intra-arterially to the brain, and intracerebroventricularly. Physiological effects were recorded while alveolar $P_{CO_2}$ was controlled at a constant level except when changes in gas tension were made in order to measure $CO_2$-ventilatory responsiveness.

**3** Adequate doses of GBTX given intravenously by bolus injection elicited a non-tachyphylactic reflex response triad of apnoea, hypotension and bradycardia mediated by the vagus nerves independently of arterial baroreceptor and chemoreceptor innervation.

**4** After vagotomy, additional amounts of GBTX (i.v.) resulted in apneustic breathing, hypertension and tachycardia. The cardiovascular effects were abolished by ganglionic blockade with hexamethonium.

**5** Smaller doses of GBTX were required intra-arterially and intracerebroventricularly than by the intravenous route of injection to produce respiratory irregularity and cardiovascular hyperactivity.

**6** Evoked motor responses, electrical excitability of the medulla oblongata and $CO_2$-ventilatory responsiveness were largely spared even though GBTX caused marked disturbances in respiratory rhythmicity and cardiovascular functions.

**7** It is concluded that GBTX acts reflexly on vagally innervated receptors to evoke a Bezold-Jarisch effect but that the toxin further acts centrally to cause irregular breathholding and hypertension with tachycardia, leading ultimately to respiratory and circulatory failure.

## Introduction

The neurotoxin produced by *Gymnodinium breve*, GBTX, is different in many respects from saxitoxin (STX) produced by certain *Gonyaulax* phytoplankton species (see LoCicero, 1975). *Gymnodinium breve* is an unarmoured dinoflagellate that generates red tides in the Gulf of Mexico whereas the saxitoxin-formers are armoured dinoflagellates responsible for the Atlantic and Pacific coastal red tides. STX, a hydrophilic substance, is concentrated by molluscs which in turn carry a lethal form of intoxication manifested by generalized paralysis in humans and seabirds unfortunate enough to have consumed the contaminated shellfish. By contrast, the fragile *Gymnodinium breve* is directly poisonous to fish in which the lipophilic neurotoxin is evidently absorbed from broken dinoflagellate cells passing through the gills. Thus, catastrophic fish kills represent the special ecological disturbance caused by red-tide blooms of the unarmoured organisms. Effects on humans are mostly confined to mucosal irritation and respiratory discomfort experienced by persons exposed to sea spray in which ruptured dinoflagellates have been aerosolized. Shellfish contamination does not constitute a significant public health hazard in connection with the *Gymnodinium breve* red-tide blooms.

The toxic reaction of vertebrate species to GBTX is a convulsive demise quite unlike the terminal paralytic depression that is caused by saxitoxin. Nevertheless, respiratory failure has been implicated in GBTX lethality as with saxitoxin. Ellis, Spikes & Johnson (1979) have described a Bezold-Jarisch reflex effect, comprising apnoea, bradycardia and hypotension

[1] Permanent address: Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, Texas 77550, U.S.A.

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induced by intravenous GBTX in dogs. The present study was undertaken to examine further this response in another species, namely the cat, and to explore possible actions of GBTX on central respiratory and circulatory control mechanisms.

## Methods

Experiments were performed on nine cats anaesthetised with pentobarbitone sodium, 40 mg/kg injected intraperitoneally. In every case, the trachea was cannulated, a vein was catheterized for drug administration and an artery was catheterized for monitoring the blood pressure. Respiratory excursions were recorded by integration of tracheal air flow measured with a No. 0 Fleisch pneumotachograph. Endotracheal $P_{CO_2}$ was measured continuously through a small sampling tube connected to a Beckman infrared analyzer. Alveolar $CO_2$ tension ($P_ACO_2$) was controlled by overflow delivery of appropriate gas mixtures of $CO_2$, $O_2$ and air from a manifold of flowmeters to the open tracheal cannula or through a positive-pressure respirator when artifical ventilation was required. Heart rate was measured by means of a tachometer triggered with the blood pressure pulse.

The following procedures were performed for specific purposes. The diaphragmatic electromyogram (EMG) was recorded through fine wire electrodes sewn into the abdominal side of the left hemidiaphragm. The signal was amplified with suitable electronic filtering by conventional means to provide an audible as well as visible output. The left phrenic nerve was exposed low in the neck and was stimulated supramaximally through a stimulus isolation unit with 1 ms biphasic pulses delivered in 2 s bursts of 50 Hz every 60 s. The medullary reticular formation was stimulated with a bipolar electrode inserted stereotaxically through the cerebellum. A submaximal current which produced sustained inspiration was delivered through a stimulus isolation unit as 1 ms biphasic pulses at 50 Hz entrained for 5 s every 100 s. GBTX was injected into the cerebrospinal fluid through a cannula placed stereotaxically into a lateral cerebral ventricle (McCarthy & Borison, 1966). GBTX was injected into the arterial blood supply to the brain through a fine catheter inserted into the lingual branch of the right external carotid artery after ligation of the left external carotid artery and occlusion of the vertebral arteries with a McDowell clamp. Dependence of the brain on oxygen delivered by the right carotid artery was confirmed by briefly occluding that vessel with resultant temporary arrest of the respiration. Transmission through a skeletal neuromuscular junction was evaluated by stimulating electrically the decentralized peroneal nerve and recording the evoked contractions of the anterior tibialis muscle. Transmission through an autonomic ganglion was evaluated by stimulating electrically the decentralized preganglionic cervical sympathetic nerve and recording the evoked contractions of the nictitating membrane. All the forms of physiological behaviour examined in this study were recorded through appropriate transducers on a six-channel, rectilinear ink-writing polygraph.

The GBTX sample we used was the preparation described by Spikes, Ray, Aldrich & Nash (1968), made available through the courtesy of Dr S. Ray. The oily substance, containing ether, was evaporated to a constant weight and suspended in a 50:50 medium of 95% ethanol and GAF emulphor 620 which was then diluted with water to make a stock solution of 10 mg GBTX per ml containing 25% ethanol and 25% emulphor. This preparation was further diluted in water at least 10 times for purposes of injection. Control injections of the suspending vehicle did not in any instance yield confounding effects.

## Results

### *Effects of bolus injections of GBTX on respiration and blood pressure (Figure 1)*

Bolus injections of GBTX in 0.4 to 1.6 ml of solution were administered intravenously at approximately 10 min intervals to an anaesthetized but otherwise neurologically intact cat. $P_ACO_2$ was controlled at 40 mmHg (5.7%). The threshold response obtained at 10 μg/kg consisted of a brief diminution in breathing amplitude with accompanying acceleration in respiratory frequency and a just detectable fall in the blood pressure. Geometric increments in the dose of GBTX evoked progressively greater and longer physiological perturbations, i.e. apnoea, bradycardia and hypotension. Nevertheless, recovery occurred rapidly following each injection with a small cumulative influence becoming evident over the full dosage range. Most noteworthy at the dose level of 160 μg/kg was the marked slowing of the respiration and the biphasic depressor-pressor effect on the blood pressure. Infusion of 250 μg/kg over a period of 8 min caused gradual respiratory deceleration and the development of hypotension without evidence of escape, indicating thereby that the bolus-evoked transient effects are terminated by rapid body redistribution of the lipophilic toxin.

### *Effects of repeated fixed bolus intravenous injections of toxin before and after vagotomy in a cat with arterial chemoreceptors and baroreceptors denervated (Figure 2)*

The dose of 40 μg/kg GBTX was injected intravenously 14 times over a period of 3 h at constant

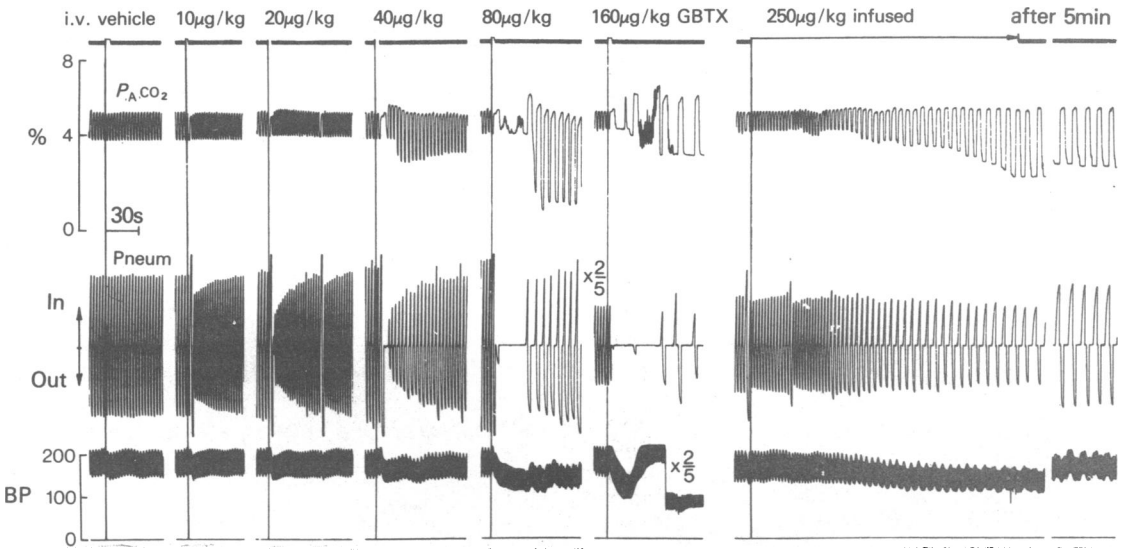


**Figure 1** Effects of i.v. dose increments of GBTX at approximately 10 min intervals on respiration and blood pressure at constant alveolar $CO_2$ tension ($P_ACO_2$). Peak level of top tracing represents $P_ACO_2$ maintained at 5.7% by controlling inspired gas concentration. Middle tracing is integrated respiratory air flow recorded by tracheal pneumotachograph (Pneum). Pen resets to zero at peak inspiration and peak expiration; breathholding is depicted as a measurable baseline space between inspiratory and expiratory excursions. Note recording sensitivity of the pneumotachograph reduced by 2/5 before injection of 160 μg/kg GBTX. Recording of blood pressure level was lowered temporarily to accommodate the secondary hypertensive response.

$P_ACO_2$. The carotid sinus nerves and the aortic depressor nerves were cut high in the neck before starting the test injections. The vagosympathetic nerve trunks were cut low in the neck after the eighth injection of GBTX. Effects were observed on respiration, diaphragm EMG, arterial blood pressure and heart rate. The phrenic nerve was stimulated periodically throughout. Tracings of $P_ACO_2$ and respiratory excursions have been omitted from Figure 2 for improved illustration. A second cat gave equivalent results.

Before vagotomy, but after arterial receptor denervation, GBTX at 15 min intervals gave no indication of tachyphylaxis over the course of 8 injections; rather, the transient response was enhanced. On the other hand, an abatement of respiratory rate, visible in the EMG tracing, occurred while the response to phrenic nerve stimulation remained unaffected.

After vagotomy, the respiration immediately converted to an apneustic pattern (irregular breathholding) at which point administration of oxygen was instituted to forestall brain hypoxia. The prompt transient apnoea, vasodepression and bradycardia were no longer elicitable by GBTX which is consistent with the view that the foregoing response triad is a Bezold-Jarisch reflex effect of the toxin, mediated by the vagus nerve. A remaining slower hypotensive effect and associated weak cardiodeceleration were then unmasked in the responses to test injections 9, 10 and 11. The nature of this effect suggests a central inhibition of sympathetic tone. Following the tenth test, the interval between injections was reduced to 10 min in order better to reveal a cumulative influence. The appearance of hypertension, cardioacceleration and generalized muscle twitching then came on rapidly. The cardiovascular response to GBTX was blocked by prior injection of hexamethonium (1 mg/kg, i.v.) attesting to its central origin, while the somatomotor excitation by GBTX persisted and the apneustic breathing as well as the phrenic-EMG response continued with no apparent change. This experiment demonstrates that longlasting generalized sympathetic and somatomotor excitation along with respiratory dysrhythmia results from central actions of GBTX.

Steady state measurements of respiratory excursions at two levels of $P_ACO_2$ were obtained before GBTX administration and again after the seventh injection. Central $CO_2$ responsiveness was unchanged after the accumulated dose of 280 μg/kg GBTX despite a reduction in respiratory frequency from 40 to 20 breaths per min that occurred over the same time.

*Intracarotid arterial injection of GBTX*

In Figure 3 is shown a comparison of effects of GBTX (40 μg/kg) injected into a carotid artery, constituting

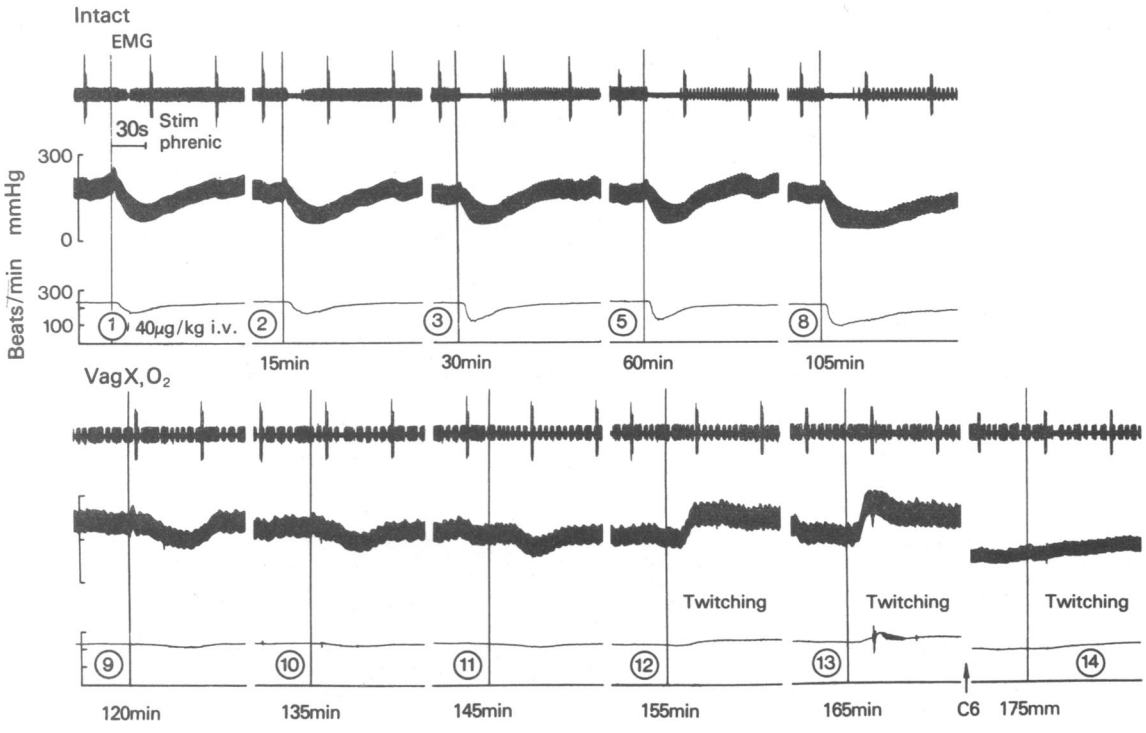


**Figure 2** Effect of 40 μg/kg i.v. GBTX injected repeatedly before and after bilateral vagotomy (Vag X) at a constant $P_ACO_2$. Encircled number indicates response position in series. Elapsed time to injection is given below respective dose delivery line. Respiration is recorded as diaphragmatic EMG with superimposed burst of phrenic-evoked potentials occurring every minute. Blood pressure and heart rate are represented in each panel. Note breathholding (apneustic respiration) evident as prolonged discharges in the EMG tracing. Hexamethonium (C6), injected between panels numbered 13 and 14, reduced the blood pressure and heart rate and prevented a subsequent cardiovascular response to GBTX.

herein the sole blood supply to the head, and into the inferior vena cava. The modified response to a bolus intravenous injection of GBTX after vagotomy was confirmed. By comparison, the effect of intracarotid injection in the vagotomized cat was characterized by quick development of apneustic breathing and a severe circulatory hypertension. These processes reverted to their pre-injection condition in a few minutes. Thus, administration of GBTX into the cranial circulation produced a temporary amplification of the central influence otherwise manifested by the toxin in much larger doses when given intravenously.

*Intracerebroventricular GBTX and electrical stimulation of the medulla oblongata*

In the experiment presented in Figure 4, a cannula had been inserted into the cat's lateral cerebral ventricle and a bipolar stimulating electrode was positioned stereotaxically in the medial reticular formation of the medulla oblongata to evoke a sustained inspiratory movement. $P_ACO_2$ was controlled at a fixed level of 40 mmHg. Increasing amounts of GBTX from 0.1 to 20 μg were injected at a constant volume of 0.25 ml into the ventricular cannula at 15 min intervals.

Responses to electrical stimulation of the reticular formation are shown in the pneumotachogram, diaphragmatic EMG, blood pressure and cardiotachogram immediately before and after the smallest dose of GBTX (0.1 μg) administered at time zero. Thereafter, evoked responses are shown at 15 min after each of the doses indicated between the panels. After repeated injections including the dose of 10 μg given over a period of one hour, GBTX produced definite slowing of respiration and heart rate as the major consequence of its entry into the brain. The respiratory and vasomotor responses to medullary stimulation, on the other hand, were only slightly affected although the previously evoked bradycardia was no

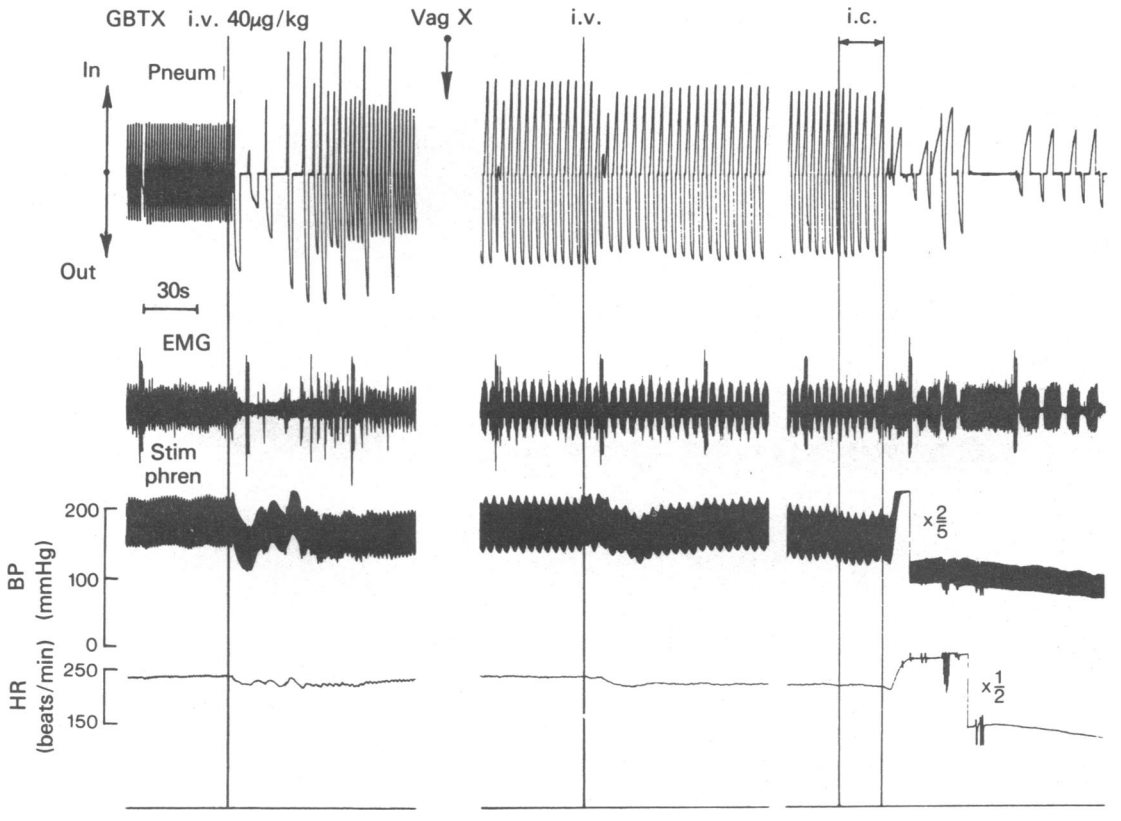


**Figure 3** Comparison of responses to intracarotid (i.c.) and intravenous (i.v.) injections of GBTX (40 μg/kg). Tracings from top down are pneumotachogram (Pneum) diaphragmatic EMG with superimposed burst of phrenic evoked (Stim phren.) potentials occurring every minute, blood pressure (BP), and heart rate (HR). Intracarotid injection via fine catheter took 27 s. Note the prompt onset of apneustic breathing, hypertension and tachycardia induced through the intra-arterial route of injection.

longer evident at the lowered working heart rate. Following the two injections of 20 μg GBTX, the respiration became apneustic and the blood pressure was elevated with accompanying cardioacceleration. Subsequent reduction of the hypertension and tachycardia by the intravenous administration of hexamethonium affirmed a central action of GBTX. Spontaneous breathing was virtually incompetent by the time the ganglionic blockade was wearing off, yet the evoked response to medullary stimulation remained largely intact. Indeed, stimulation of the inspiratory centre was later used as a mode of artificial ventilation when complete respiratory failure had supervened. Thus, following a total intracerebroventricular dose of approximately 50 to 100 times less GBTX than required systemically, respiratory rhythmicity was arrested even while the motor integration of breathing remained operational when driven electrically.

*Effects of intravenous GBTX on skeletal neuromuscular and autonomic ganglionic transmission*

In a cat prepared for recording contractions of the anterior tibialis muscle and the nictitating membrane as indicators of neuromuscular and ganglionic impulse transmission, GBTX was administered over 3 h in divided doses reaching a total of 1500 μg/kg at the time of death. Figure 5 shows the effects of the first, last and four intermediate doses. The vagus nerve had been sectioned on the left side where the cervical sympathetic nerve was being stimulated. Artifical ventilation was given from the outset to maintain a constant $P_ACO_2$ in anticipation of the certain failure to occur in the respiratory control system. The ventilation pump was stopped at intervals to test for spontaneous respiratory capability. Breathing became apneustic at an accumulated dose of 500 μg/kg GBTX and the respiration ceased altogether after 900 μg/kg had been

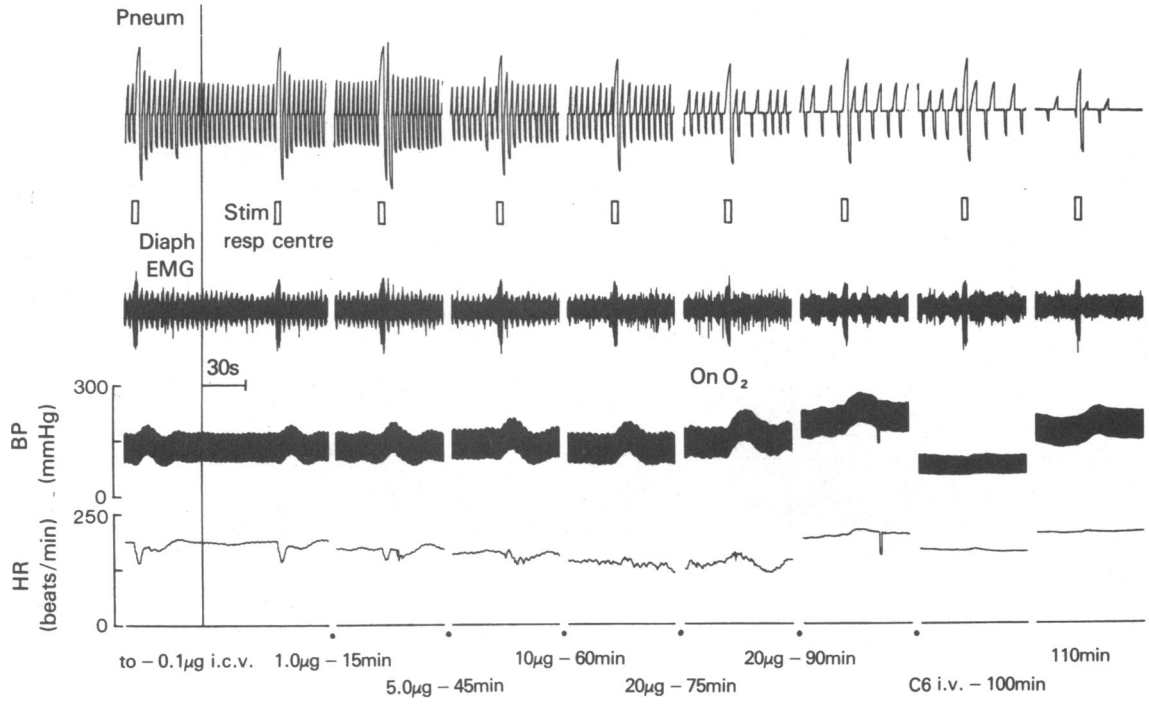


**Figure 4** Effect of intracerebroventricular GBTX on spontaneous breathing and on the electrical excitability of the respiratory centre at constant $P_ACO_2$. Recorded variables as in Figure 3 except that evoked burst activity in the EMG was initiated in the medulla oblongata. Note electrically induced responses also in the pneumotachogram (Pneum) and cardiovascular recordings. GBTX was administered in 0.25 ml solution at every level. Except for the first injection (0.1 μg) made on the delivery line at zerotime ($t_0$), all subsequent injections of GBTX were given between panels at the elapsed times indicated. Note sparing of the electrically evoked responses while breathing became apneustic and hypertension and tachycardia developed from i.c.v. GBTX. Hexamethonium (C6) injected i.v. at 100 min temporarily reduced the blood pressure (BP) and heart rate (HR).

injected. This experiment is noteworthy in the following respects. Effects on the blood pressure are consistent with the previous results. Except for shifts in the baseline, the nictitating membrane continued to respond to preganglionic nerve stimulation with little attenuation. At 110 min, myoclonic jerks of the head are evident as line artifacts in the membrane tracing. The tibialis muscle showed no sign of weakening until the very end. Indeed, facilitation of contraction strength became evident simultaneously with the development of circulatory hypertension suggesting that the same cellular excitatory mechanism was operating in both processes. On the other hand, the cardiovascular hyperactivity must be attributed to a central action of GBTX since the sympathetic neuroeffector test object showed no increase in responsiveness to preganglionic electrical stimulation. Most remarkable is the fact that neuromuscular paralysis was never achieved before death which resulted from circulatory failure. Confirmatory results were obtained in a second cat.

## Discussion

### *Reflex effects of GBTX*

The 'Bezold-Jarisch effect' was originally described by Bezold in 1862 as a cardiovascular response, i.e. bradycardia and hypotension, to intravenous injection of the veratrum alkaloids acting on vagal afferent receptors in the heart (see Krayer, 1961). Cramer (1915) later characterized the respiratory component of the veratrum-induced reflex response which he attributed correctly to excitation of pulmonary receptors. It must be emphasized that the afferent nerves mediating the entire reflex pattern were shown to be contained in the vagus nerve trunk separately from the aortic depressor nerve. Since then, a diversity of substances have been found which mimic the Bezold-Jarisch effect but which may also yield more complex responses including secondary tachycardia, hypertension, tachypnoea and mixed combinations resulting from actions on widely distributed systemic

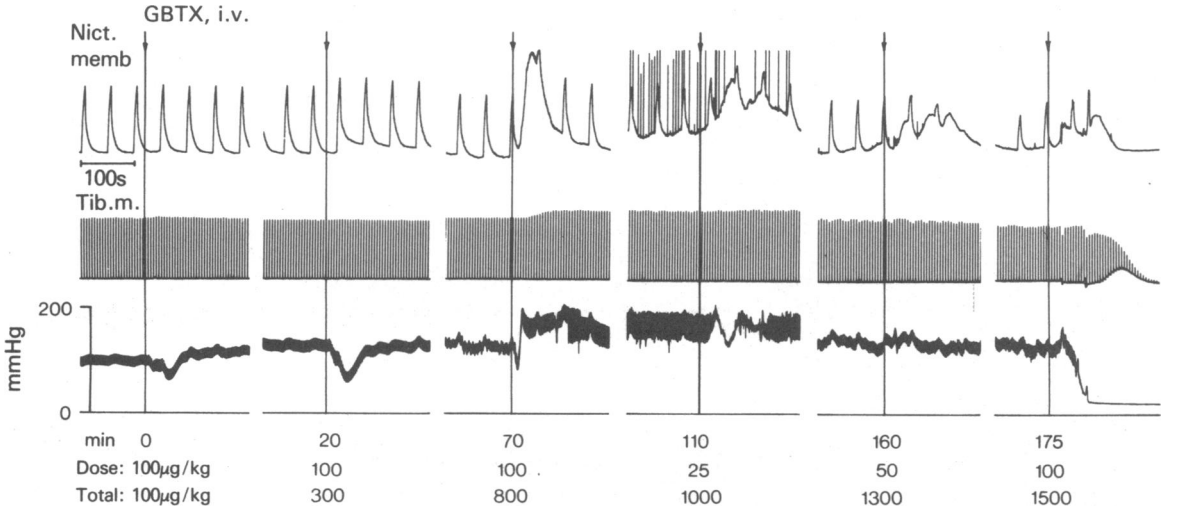


**Figure 5** Effects of GBTX on contractions of the nictitating membrane (Nict. memb.) evoked by supramaximal stimulation of the preganglionic sympathetic nerve and of the anterior tibialis muscle (Tib. m.) evoked by supramaximal stimulation of the peroneal nerve, at a constant $P_ACO_2$. Selected injections are shown on the delivery lines at the indicated elapsed times up to 175 min and a total cumulative amount of 1500 μg/kg GBTX to the point of cardiac arrest. Note mild impairment in the evoked neuroeffector responses occurring after 1000 μg/kg had accumulated systemically. Enhancement of the tibialis contraction was produced earlier at the time that gross hypertension first appeared in the blood pressure tracing. The actual forces developed by the contractions of the nictitating membrane and the tibialis muscle were not measured.

mechanoreceptors and chemoreceptors (Dawes & Comroe, 1954).

The particular neuroexcitatory effectiveness of the veratrum alkaloids lies in their ability to cause impulse repetition through alteration of membrane repolarization kinetics (Blackman, Borison & Milne, 1975). A similar action has been described for GBTX (Westerfield, Moore, Kim & Padilla, 1977). In the present work, therefore, it is not surprising that the Bezold-Jarisch response triad evoked by intravenous GBTX persisted after the nerves of Hering (carotid sinus n.) and of Cyon and Ludwig (aortic depressor n.) had been sectioned, and that it was subsequently abolished by interruption of the vagus nerve trunks. However, the residual and cumulative non-vagal influence of GBTX of the respiratory and cardiovascular systems still required an explanation.

*Central effects of GBTX*

Three lines of evidence support a selective central depressant action of GBTX on the respiratory rhythm generator: (1) Apneustic respiration was produced by intravenous, intracarotid and intracerebroventricular administration, in the latter instances without the benefit of vagotomy. No mechanism is known by which an isolated drug action at a peripheral locus can produce such a derangement in respiratory pattern. (2) Tidal volume responsiveness to alterations in $P_ACO_2$ was not affected in the presence of a prominent bradypnoea caused by GBTX. (3) Motor pathways and the effector muscles of respiration continued to respond to electrical stimulation of the medullary reticular formation after spontaneous breathing had been disrupted by the injection of GBTX into the cerebrospinal fluid.

It is thus evident that two processes affecting the control of respiration, peripheral and central, proceed simultaneously during systemic accumulation of GBTX. No indication was obtained of tachyphylaxis occurring in the vagal reflex on repeated intravenous bolus injections of GBTX, and the response to its intravenous infusion grew progressively. Hence, we have reason to believe that the peripheral transient effects reflect redistribution kinetics and that a definite cumulative effect did occur peripherally despite rapid movement of the lipophilic toxin between body fluid compartments. After vagotomy, apneustic respiration was well sustained during dose multiplication of GBTX. It is thus apparent that while the rhythm generator showed early vulnerability to GBTX resulting in irregular respiration, $CO_2$ sensitivity and amplitude of breathing were not easily depressed. Nevertheless, inadequate pulmonary ventilation caused by GBTX acting centrally results ultimately in the asphyxial arrest of breathing. This picture is to be

contrasted with the central respiratory effect of saxitoxin which is manifested as a simultaneous depression of rhythm, amplitude and $CO_2$ sensitivity (Borison & McCarthy, 1977).

Development of arterial hypertension and tachycardia also reflected central accumulation of intravenous GBTX. After vagotomy, this central influence, operating through sympathetic discharge, yielded massive monophasic pressor responses and tachycardia leading to cardiac failure. Elicitation of this effect with small doses of GBTX administered intracerebroventricularly and its reversal with intravenous hexamethonium further substantiate a central action of GBTX on the cardiovascular control mechanism.

*Junctional transmission spared by GBTX*

An outstanding feature of the present work was that all indicators of junctional transmission through evoked motor activity, whether initiated peripherally or centrally in somatic or autonomic neural pathways, showed little or no blockade of nerve-to-nerve or nerve-to-muscle impulse transfer. Indeed, enhancement in the indirectly-elicited tibialis muscle twitch response is best explained as resulting from repetition of impulse delivery at the nerve terminal. This also accounts for fasciculations observed in the tongue at the time that the evoked muscle twitch was most augmented. The subsequent generalized synchronous myoclonic jerks were probably generated in the spinal cord. Where detectable depression in evoked motor responses did occur after large whole body levels of GBTX had been achieved, this late effect cannot be assigned to any single influence on conduction, transmission, contraction or metabolism.

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