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ISOLATION AND IDENTIFICATION OF TOXIC FRACTIONS OF COBRA VENOM

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Three major physiological changes are observed following the injection of an intravenous lethal dose of crude cobra venom into anesthetized dogs or monkeys. The initial change appears to be a complete and irreversible loss of cortical electrical activity, which occurs within 30-60 sec (1). A depression of the electroencephalogram (EEG) is followed, within 3-5 min, by a sharp rise in portal venous pressure and a precipitous fall in systemic blood pressure (2,3). Both pressures return to near normal levels in approximately 30-60 min and remain unchanged for from 4-6 hr. At this time progressive respiratory difficulties became apparent, ultimately terminating in apnea (4). This respiratory paralysis has been shown by some (5,6) to be due to blockade at the neuromuscular junction of the diaphragm. Artificial ventilation at time of apnea extends survival time; however, all animals ultimately expire (4).

The complex nature of the physiologic changes observed with the crude cobra venom prompted this study which included the fractionization of the venom and characterization of the specific pharmacologic activity associated with each fraction.

MATERIALS AND METHODS

Sixty adult mongrel dogs, anesthetized with pentobarbital sodium, 30 mg per kg, were used in this study. Arterial blood pressure was continuously monitored using a polyethylene catheter inserted into the femoral artery and connected to a Statham pressure transducer and an E and M Physiograph recorder. Portal venous pressure was recorded via a catheter inserted into the splenic vein and advanced into the portal circulation.

Respiratory rate, electrocardiogram (ECG), and heart

rate were monitored using a pair of needle-tip electrodes
placed on either side of the chest wall and connected to the
E and M Physiograph.

The EEG was recorded via four unipolar silver
electrodes placed directly on the dura of each hemisphere of
the brain and connected to Hi-gain preamplifiers. Two of the
paramedial electrodes were placed in the frontal and two in
the occipital areas of the brain. Continuous recordings were
made on a model 5 Grass polygraph.

Crude cobra venom, Naja naja, was used in the study.
Initial toxicity of the venom was determined by a time-to-death
mouse assay. Chromatographic separation of the venom was
accomplished on Carboxymethyl Sephadex C-25 in 25mm x 300mm
dimension column. In some cases the chromatography was preceded
by dialysis against distilled water, using a Visking cellophane
bag in a closed container, which was inverted twice per minute.
Samples of venom were dissolved in 0.01 M phosphate buffer,
pH 6.0, and applied to the column. Elution was carried out with
a gradient in pH and salt concentration to a final pH value of
8.0 and 0.4 M phosphate. Well defined peaks in the u.v. absorp-
tion were obtained from this chromatogram. The fraction which
came through the column was dried, freed of salt and rechroma-
tographed on diethylaminoethyl (DEAE)-Sephadex, using a
gradient elution in phosphate to a final concentration of 0.06
M, pH 6.50.

When subjected to dialysis and chromatography, the
crude venom yielded a total of 12 separate peaks. All samples
were stored at approximately 5° until time of use. Just prior
to injection the individual fractions were allowed to return
to room temperature.

Following an appropriate control period, dogs were
given an intravenous injection of one of the 12 fractions.
Injection of the phosphate buffered venom fractions was made
over a 3-5 min period using a solution containing 1 mg per ml.
Graded injections were made on a mg per kg basis to determine
the minimum lethal dose for each fraction. The 60 dogs used in
this study were divided into the following groups:

> Group I...Seventeen animals administered 0.5 mg
>   per kg of fraction 1.
> Group II..Thirty animals administered 0.5 mg per
>   kg of fractions 2-11.
> Group III.Thirteen animals administered 0.5 mg per
>   kg of fraction 12.

RESULTS

Group I.- The intravenous administration of fraction
1 produced a change in the EEG much like that previously
observed with crude venom, complete cessation of activity
occurring within 3-5 min following injection (Fig. 1A). A

slight transient fall in arterial blood pressure was also observed 5-15 min post injection, returning to control or near control level at approximately 30 min post venom. This change in blood pressure was not statistically significant (p>0.05). No change in respiratory rate, ECG or heart rate was observed following the injection of fraction 1.

Group II.- The injection of fractions 2-4,9-11 did not produce any change in the physiological parameters measured. In contrast, fractions 5-8 resulted in respiratory paralysis within 30-120 min post injection. Figure 1B shows an actual tracing representative of the progressive respiratory depression produced by these fractions. No change in ECG or blood pressure was noted after the injection of these fractions until severe respiratory depression became apparent. At this time arterial blood pressure, ECG, heart rate and EEG recordings reflected abnormalities indicative of inadequate tissue oxygenation. These changes ultimately led to an anoxic-induced cardiovascular collapse. Artificial ventilation at the time of respiratory arrest significantly extended time to death (p<0.05), but did not prevent the ultimate lethal outcome. In ventilated animals average survival time was increased from 120 min to 185 min.

Group III.- The intravenous administration of fraction 12 produced a marked effect on cardiovascular function. Immediately following injection a fall in arterial pressure occurred followed by partial recovery within 30-60 min and, finally, by a slow progressive decline terminating in death at approximately 90-150 min (Fig. 1C). No significant change in the EEG or respiratory function was noted with the administration of this fraction. Alteration of the ECG and heart rate were noted, however, at varying intervals post injection, and occurred simultaneously with the change in blood pressure.

DISCUSSION

The results of this study indicated that six physiologically active fractions can be separated from crude cobra venom by fractionization procedures. The administration of the first fraction (Peak 1) produced a marked depression of the cortical electrical activity. It has been shown that crude cobra venom contains large amounts of phospholipase A (7,8). Although it has not been established at the present time it is felt that the change in EEG may not be the result of phospholipase A activity. Preliminary studies in this laboratory have indicated that the removal of the enzyme from Peak 1 did not alter EEG activity. The loss of cortical electrical activity noted in this study, therefore, may not be explainable on a on a specific enzyme-related basis. Furthermore, the change in EEG occurred so rapidly that involvement of endogenous neurohumoral agents, i.e. histamine, serotonin, catecholamines

and bradykinin, is thought unlikely. Ferrer et al. (9) reported, however, high radioactive counts in the cerebrospinal fluid following intravenous injection of labelled venom. This penetration of the brain by the venom or venom components could account for the change observed in cortical electrical activity.

Fractions 5-8, which may produce respiratory paralysis by blocking neuromuscular transmission at the diaphragm, appeared to do so by interfering with some action of acetylcholine (10). There are, however, others who indicated that this phenomenon may be the result of increased nerve membrane permeability (11). This concept is consistent with a proposed general breakdown of membrane phospholipid by venom, producing an over all depolarization and block rather than any specific inhibition. In either case the concensus is that death following the administration of these fractions or of the crude venom was due primarily to peripheral respiratory failure. This is not the case with rattlesnake venom (Crotalus atrox) which does not appear to affect the peripheral nerves or neuromuscular transmission (12). An extension of these actions (neuromuscular block or nerve membrane depolarization) to other areas of the body may be a possible explanation of the inadequacy of artificial ventilation in preventing the ultimate lethal action of crude cobra venom (4).

The effect of fraction 12 on arterial blood pressure and heart rate indicated that cobra venom has a definite action on the cardiovascular system of the dog. Previous studies using isolated perfused heart preparations have shown that cobra venom acts on the heart only in the presence of blood. Hearts perfused with oxygenated dextran showed no toxic changes even after the administration of large doses of cobra venom. This is in contrast to rattlesnake venom, which produced marked deterioration of the isolated heart perfused with either whole blood or dextran (13). The deterioration of the cardiovascular system after the injection of fraction 12 may be due to a generalized action on the peripheral vasculature. Loss of vascular integrity and hemorrhage into the tissue has been reported (14). This action may be due to the detrimental effects of phospholipase A and/or lysolecithin on the integrity of the red blood cell membrane and the capillary endothelium (15-17).
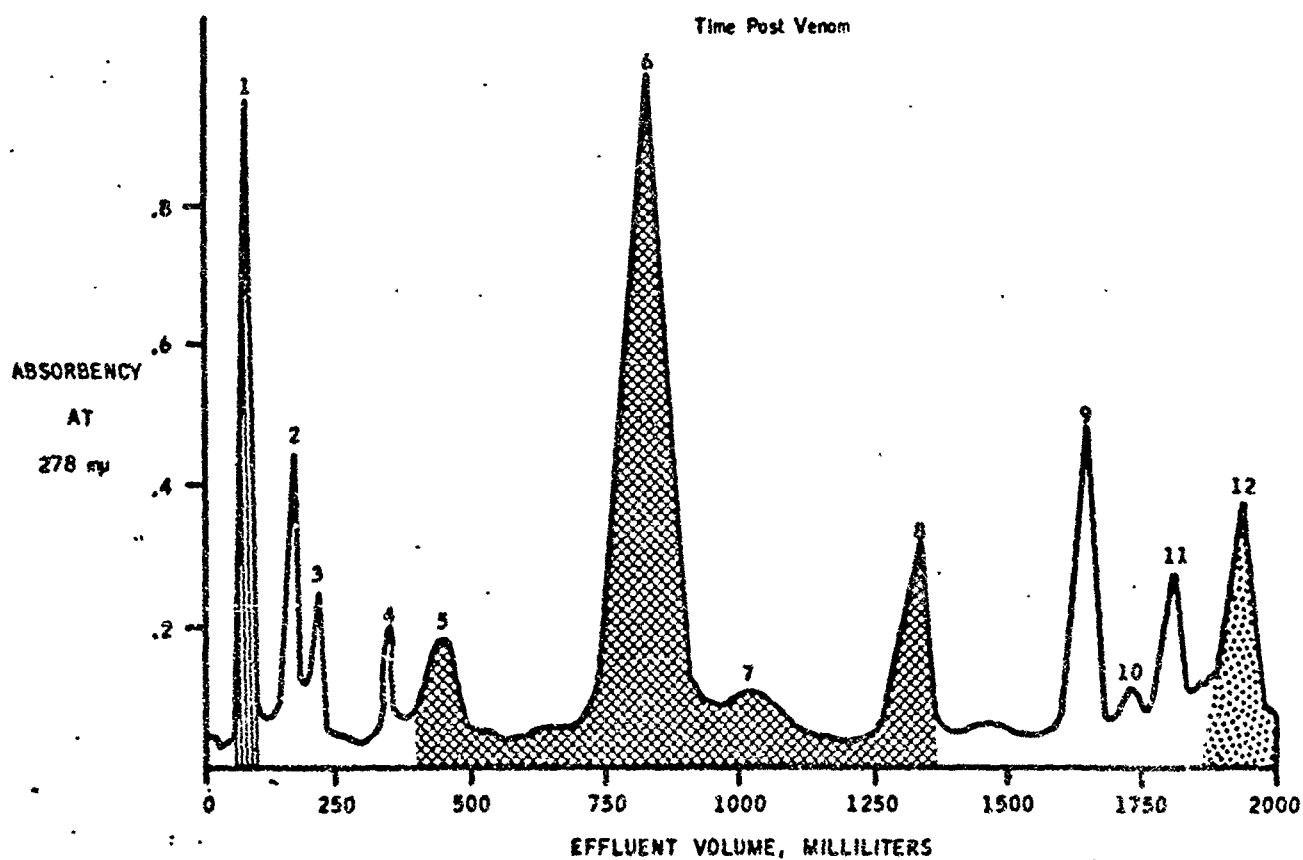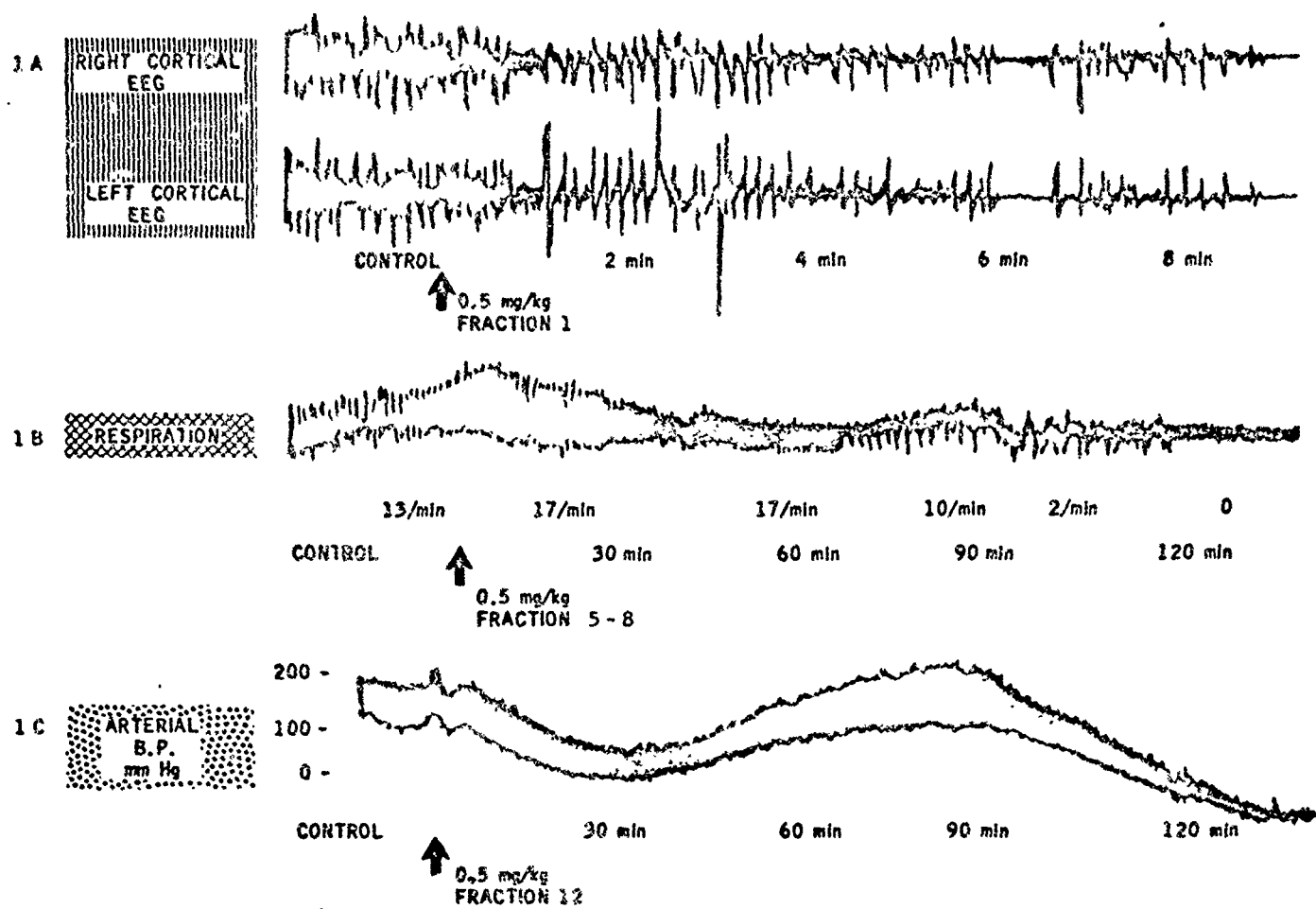
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1 A RIGHT CORTICAL EEG

LEFT CORTICAL EEG

CONTROL   2 min   4 min   6 min   8 min

0.5 mg/kg
FRACTION 1

1 B RESPIRATION

13/min   17/min   17/min   10/min   2/min   0

CONTROL   30 min   60 min   90 min   120 min

0.5 mg/kg
FRACTION 5 - 8

1 C ARTERIAL B.P. mm Hg
200 —
100 —
0 —

CONTROL   30 min   60 min   90 min   120 min

0.5 mg/kg
FRACTION 12

Time Post Venom

ABSORBENCY AT 278 mµ

EFFLUENT VOLUME, MILLILITERS

VICK, CIUCHTA, BROOMFIELD, CURRIE
and MANTHEI

LEGEND FOR ILLUSTRATION

Figure 1.- Identification of the Fractions of Crude Cobra Venom
and the Characterization of Their Physiologic Activity.

(1A). -Effect of fraction 1 (0.5 mg per kg) on cortical
electrical activity of the anesthetized dog. Complete or
near complete loss of EEG is observed at from 6 to 8 min
post injection.

(1B). -Progressive respiratory depression produced by intra-
venous injection of one of the following fractions-5,6,7
or 8. Fractions 2-4 and 9-11 produced no effect on res-
piration, EEG or blood pressure.

(1C). -Unique effect of fraction 12 on arterial blood
pressure. A fall in pressure is noted immediately post
injection. This is followed by recovery to near normal
and a subsequent decline leading to death at approximately
120 min.