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Pharmacology of *o*-chlorobenzylidene malononitrile (CS)

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Summary

1. The effects of *o*-chlorobenzylidene malononitrile (CS) have been studied on several isolated organs and tissues, anaesthetized animals and cat *encéphale isolé* preparations.

2. On the isolated guinea-pig ileum an initial dose of CS produced a small, non-maintained contraction. Subsequent doses had reduced effects. There was no effect on peristalsis when the substance was given intraluminally.

3. No significant effects of CS were detected on the rat phrenic nerve-diaphragm preparation, the isolated perfused rabbit heart or on the contractor response of the indirectly stimulated cat tibialis muscle.

4. In the cat *encéphale isolé* preparation 1 mg/kg (i.v.) produced a brief period of electrocortical alerting but no abnormal activity in the electrocorticogram. Doses in excess of 10 mg/kg produced cortical depression.

5. Intravascular injection into the chloralose anaesthetized cat resulted typically in a pressor response accompanied by a brief period of apnoea. The threshold dose for the pressor response varied with the route of administration, but generally lay between 2·5 and 12·5 μg/kg; the threshold dose for apnoea was slightly higher. Small variations in this pattern of response were seen with different species and other anaesthetics.

6. When administered by stomach tube to chloralose anaesthetized cats, CS produced no measurable effects at doses of up to 100 mg/kg.

7. No changes in blood pressure or respiration were detected in anaesthetized cats given pure CS aerosol for 1 h in concentrations of between 345 mg/m^3 and 1·39 g/m^3 via a tracheal cannula or through the upper respiratory tract. Pure CS solution given by slow intravenous infusion at a similar dose and over a similar period produced significant effects on blood pressure and respiration.

8. Pyrotechnically generated (grenade) CS produced variable effects when given by inhalation in concentrations of between 460 and 1,040 mg/m^3 for 1 hour. Respiratory depression, possibly reflex in nature, regularly occurred when the material was given via the upper respiratory tract, and respiratory stimulation occurred when it was given via a tracheal cannula.

9. Some cats were pre-exposed to a dose of 500 (mg/min)/m^3 on 4 successive days and on the fifth day anaesthetized and exposed to high concentrations of grenade CS. Three out of six cats died during or after this final exposure compared to one out of six among animals not so pre-exposed. The general pattern of response to the final exposure to CS in the two groups was similar.

Introduction

o-Chlorobenzylidene malononitrile (CS) was first prepared in 1928 by Corson &

Stoughton who reported that it had irritant properties. In 1958 it was introduced as the active constituent of a riot control device to replace ω-chloroacetophenone (CAP, CN) and since then there have been reports of various aspects of its toxicology and of its effectiveness in man. Punte, Weimer, Ballard & Wilding (1962) reported on its intravenous and inhalation toxicities to several species of animals, and Ballantyne & Callaway (1972) describe the toxic and pathological effects of exposing various species to very high concentrations of CS grenade smoke.

The only pharmacological study appears to have been that of Biscoe & Shephard (1962) who described the responses in the cat and the dog to the intravascular injection of ethanol solutions of CS. Their conclusion was that the substance was acting as a non-specific irritant stimulating at a variety of sites. Interpretation of their results was, however, somewhat complicated by the fact that the ethanol, used as solvent for CS, itself exerted some pharmacological actions at the doses used.

The study reported here was designed to investigate a variety of possible pharmacological effects of CS when administered by various routes to whole animals and when applied to isolated organs and tissues. In practical circumstances the substance is most likely to enter the body via the respiratory tract therefore much of the work reported deals with its effects when administered by inhalation. There is also the possibility that the substance will be swallowed so its pharmacological effects on the gut are also reported.

This also seemed of relevance in view of reports of a small incidence of diarrhoea after exposure to CS (Report of the *Enquiry into the Medical and Toxicological Aspects of CS*, Pt. II, HMSO, 1971).

Methods

Isolated organs and tissues

Isolated guinea-pig ileum

(a) Contractions of longitudinal muscle were recorded using standard methods. CS was dissolved in ethanol and introduced into the 5 ml bath in a volume of 0·005 ml to give a final solvent concentration of 0·1%. This concentration of ethanol did not produce contractions of the ileum or modify acetylcholine or histamine-induced contractions.

(b) The effects of CS on the peristaltic reflex were studied using the method of Trendelenberg essentially as described by Burn (1952). The peristaltic reflex was elicited every 5 min by increasing the intraluminal pressure by 3–4 cm water for 60 seconds. In one series of experiments intraluminal pressure was maintained at 4 cm water throughout.

To study the actions of drugs acting from the serosal surface they were added to the bath fluid 1 min before raising the intraluminal pressure. The actions of drugs from the mucosal surface were studied by introducing them into the lumen through a polythene cannula inserted into the glass J tube to which the anal end of the ileum was tied.

CS was dissolved in ethanol and given in a volume of 0·05 ml to give a final solvent concentration of 0·1%. This concentration of ethanol alone had no effect on the peristaltic reflex.

For intraluminal injection CS was given in a solution of 0·1% ethanolic Ringer-Tyrode. Five millilitres of the solution were injected into the lumen over 2 min with the excess solution being bled off through a side arm from the J tube.

Isolated rat phrenic nerve-diaphragm preparation

The preparation was set up as described by Bulbring (1946). Solutions of CS were prepared and added to the bath as described in the isolated guinea-pig ileum experiments.

Isolated perfused rabbit heart

The preparation was set up as described by Burn (1952). Solutions of CS in polyethylene glycol (PEG 300) were prepared at a concentration of 35·37 mg/ml and diluted 1:1,000 in Ringer-Locke solution immediately before use to give a 2×10^{-4}M solution, the limit of aqueous solubility, 5 ml of this solution were injected over a period of 1 min into the coronary circulation.

Experiments on whole animals

Anaesthetized cats, dogs and rabbits. CS given by intravascular injection, inhalation and by stomach tube

Experiments were performed on cats, dogs and rabbits. In most experiments anaesthesia was achieved as follows. Cats were induced with ether and maintained on chloralose (75–80 mg/kg) given by slow intravenous injection into the cephalic vein of the forelimb. Dogs were premedicated with 1 mg/kg morphine hydrochloride given 1 h before an intravenous infusion of chloralose. The initial dose of chloralose was 0·1 g/kg and anaesthesia was maintained with doses of 20 mg/kg administered intravenously approximately every 30 minutes. Rabbits were anaesthetized with urethane (1·8 g/kg) given into the ear vein.

In some experiments other anaesthetic procedures were used. These are detailed in **Results.**

For intravascular injection of CS solutions polythene cannulae were used. One was passed from the right femoral vein to the right atrium and a second was passed along the left femoral artery to the aortic arch. The positions of the tips of these cannulae were confirmed by postmortem examination. In many animals injections were also made into the carotid artery by way of a T cannula and into the femoral vein through a short polythene cannula. In some cats CS solution was given by slow infusion into the femoral vein. In a few experiments metabolites of CS were given by intravenous injection.

Systemic blood pressure was recorded from the femoral artery using a Statham Physiological Transducer Model P23AA (1 mmHg≡1·333 mbar). The respiratory rate was recorded using an Impedance Pneumograph (E & M) employing subcutaneous needle electrodes on either side of the thorax. The electrocardiogram (ECG) was also recorded from needle electrodes similarly placed.

In some animals tidal volumes were measured using a screen flowmeter. Pressure changes across the screen were measured using a Statham transducer

Model No. PM97. All signals were amplified and displayed on a pen recorder (Physiograph 'Six', E & M Instrument Co. Inc.).

In the inhalation experiments anaesthetized cats were exposed to either pure or pyrotechnically generated CS (grenade CS) for periods of 1 hour.

In the pure CS experiments aerosols were generated from suspensions of CS in 10^{-4}M acetic acid using a Collison spray. The air pressures and suspension concentrations were varied to suit required conditions. The CS was wet milled in a ball mill to a suitable particle size (approximate average diameter 2 μm), diluted to the required concentration and sprayed, with stirring, continuously. Samples were taken at intervals and concentration estimated by measurement of optical density in a spectrophotometer at the required wave length in acid alcohol. In some cats the aerosol was inspired through a Y cannula inserted in the trachea. Expiration was through a screen flow meter to enable tidal volumes to be recorded. The inspiration/expiration phase was regulated by means of a pressure sensitive pick-up controlling two solenoid valves. Other cats received the aerosol via the upper respiratory tract. In these experiments the nose and mouth regions protruded through a rubber diaphragm into a tube through which the aerosol was drawn.

In the grenade CS experiments scaled down pyrotechnic devices to simulate CS grenades were used. These usually contained 0·5 g CS and 1·5 g of standard pyrotechnic mixture. The grenades were ignited in a 1 m^3 chamber at intervals of 7·5 min with stirring. Samples were taken of the chamber atmosphere at such times as to coincide with the minimum and maximum concentrations and estimated spectrometrically as described above. The chamber was exhausted continuously past the animal's nose (upper respiratory tract) or by the previously described method using a Y cannula (lower respiratory tract) at approximately 50 l./minute. This gave a concentration-decay curve within the chamber such that the mean concentration throughout the 1 h exposure period was significantly constant. The dose of CS to which cats were exposed by inhalation was expressed as Ct (concentration × time in $(mg/min)/m^3$).

A few experiments were carried out using half sized grenades containing 0·25 g CS. In these experiments where lower concentrations of CS were required the grenade products which emerged from the ignition chamber were diluted with air introduced through a side-arm. Some control experiments were performed using grenades containing no CS to examine the effects of pyrotechnic mixtures alone.

In some experiments cats were pre-exposed to pure CS ($Ct=500$ $(mg/min)/m^3$) on the 4 days before an exposure to a high concentration as described above.

In addition to the recordings of respiratory and cardiovascular parameters, in the grenade CS experiments arterial blood samples were obtained at intervals for the analysis of blood gases. Oxygen tensions were obtained using a Clarke Oxygen Electrode type E5046 and carbon dioxide tensions were obtained by the interpolation technique of Siggaard-Anderson, Engel, Jørgenson & Astrup (1960) using an Astrup semi-micro pH electrode type G297/G2 connected to a Model 22 pH meter. The nomogram used was that constructed by Siggaard-Anderson & Engel (1960).

In a few experiments CS solution was given to anaesthetized cats by stomach tube. These cats had been deprived of food for 18 hours. Volumes of 2 ml/kg of solutions in PEG300 were used.

Effects on cat tibialis anterior preparation

Cats of about 2 kg were anaesthetized with chloralose (80 mg/kg) intravenously and twitches and tetani of the tibialis anterior muscle were recorded. CS dissolved in PEG300 was administered into the femoral vein.

Cat encéphale isolé *preparation*

Cats of 1·8–2·5 kg were used. The animals were prepared under halothane anaesthesia, using the operative procedure described by Bradley & Key (1958). The effects of CS on the electrocortical activity and behaviour of the preparation were studied using the method described by Brimblecombe, Green, Aldous & Thompson (1971). CS as a solution in PEG300 was given through a cannula in the femoral vein, in a volume of 0·05 ml/kg. Incremental doses ranging from 10 μg/kg to 10 mg/kg (common ratio 10) were given at 20 min intervals.

Source of compounds

The CS used in these studies together with its three metabolites, *o*-chlorobenzaldehyde, 1-(*o*-chlorophenyl) 2(dinitrile)ethane and malononitrile were synthesized in these laboratories. All other drugs used were purchased from commercial sources.

Results

Isolated organs and tissues

Effects of CS on longitudinal muscle of isolated guinea-pig ileum

A concentration of 10^{-4}M CS produced a small contraction which was not well maintained; the response declined to normal baseline level within 1 min of the addition of CS to the bath. Submaximal contractions produced by $2{\cdot}5 \times 10^{-7}$M acetylcholine and histamine added to the bath 1 min after 10^{-4}M CS were reduced in height by approximately 50%. After washing out the CS from the bath contractions induced by acetylcholine and histamine were restored to their normal levels.

Serial additions of 10^{-4}M CS to the bath at 10 min intervals, and in one preparation at 1 h intervals, produced progressively smaller responses.

Atropine sulphate (0·3 μg/ml) and morphine sulphate (1–4 μg/ml) blocked the contractor effect of 10^{-4}M CS on the ileum.

10^{-5}M CS produced a non-maintained contraction in two out of four preparations. Subsequent additions of CS at this concentration failed to produce any response.

10^{-6}M CS had no effect on the ileum.

Effects on the peristaltic reflex

The effects of 10^{-6}, 10^{-5} and 10^{-4}M solutions of CS acting from the serosal surface are shown in Fig. 1.

10^{-6}M CS had no effect. When the peristaltic reflex was elicited 1 min after the addition of 10^{-5}M CS to the bath a slight facilitation occurred followed approximately 45 s later by a depression of circular muscle contractions; this concentration did not affect the longitudinal muscle response. Subsequent peristaltic responses

elicited at 5 min intervals were similar to control responses even though the CS had not been washed out of the bath.

10^{-4}M CS produced a slight depression of the longitudinal muscle response and severe depression of circular muscle contractions. Repeated washing only partially restored peristalsis after this concentration of CS.

The ganglion-blocking drug hexamethonium (5×10^{-5}M) abolished the peristaltic waves of the circular muscle leaving the longitudinal muscle response unaffected. 10^{-5} or 10^{-4}M CS did not produce any contraction of circular muscle after blockade by hexamethonium but transiently increased the tone of the longitudinal muscle.

When injected intraluminally at a concentration of 10^{-4}M, CS did not affect the peristaltic reflex. It was also injected at a concentration of 4×10^{-4}M which exceeds maximum solubility and so the CS was in the form of a fine suspension. This, again, had no effect on the peristaltic reflex.

When the intraluminal pressure was raised by 4 cm of water for approximately 3 h the ileum showed peristaltic waves which were at first regular but later consisted of single or repetitive waves spaced at irregular intervals (Feldberg & Lin, 1949). Beleslin (1969) reported that once this irregular activity appeared the ileum was very sensitive to drugs that stimulate peristalsis. With the ileum subjected to such a rise in intraluminal pressure, 10^{-5}M CS in the bath partially restored persistalsis for approximately 30 s whereas 5×10^{-7}M carbachol added to the bath after CS produced marked restoration of the peristaltic reflex.

Isolated rat phrenic nerve-diaphragm preparation

CS at a bath concentration of 10^{-4}M had no effect on single twitches or tetani produced by direct or indirect stimulation of the muscle.

Isolated perfused rabbit heart

CS, in a total dose of 480 μg produced small, irregular changes in both rate and amplitude of beat. However, PEG300, diluted 1 to 1,000 in Ringer Locke, produced essentially identical results so it appears that CS had no effect on the preparation.

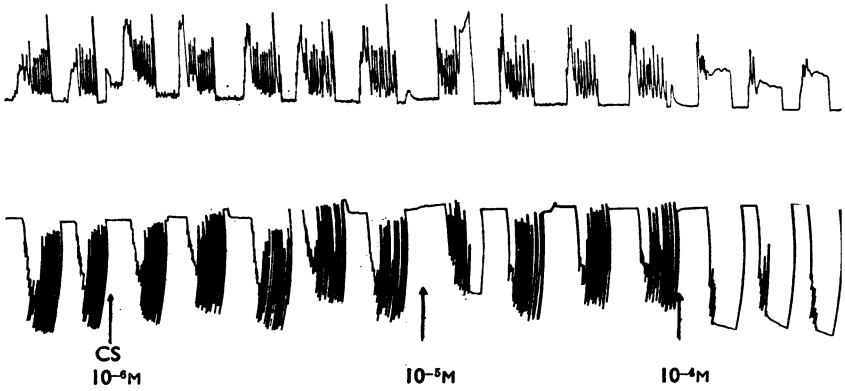

FIG. 1. Effects of CS, acting from the serosal surface, on peristalsis of the guinea-pig ileum. In this figure the upper trace shows contractions and relaxations of longitudinal muscle and the lower trace intraluminal volume changes, with increases in volume shown by downward movements.

Whole animal studies

Intravascular injection

In cats anaesthetized with chloralose the typical response to CS was a rise in arterial blood pressure with a tachycardia and increase in pulse pressure, accompanied at higher doses (312 μg/kg–1·56 mg/kg) by a brief period of apnoea which was usually followed by a period of respiratory stimulation when minute volumes were increased by 50 to 100%. This typical response is shown in Fig. 2. The dose required to produce this effect varied with the route of administration. Progressively higher doses were required to produce comparable responses when CS was given via the carotid artery, aortic arch, right atrium and femoral vein respectively. This

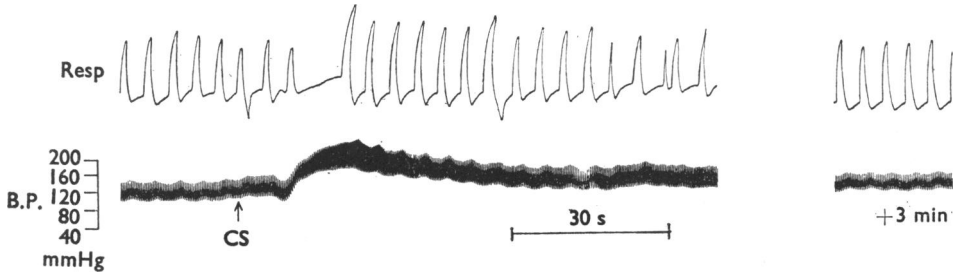

FIG. 2. Effects of intravenously injected CS (100 μg/kg) on the arterial blood pressure and respiration of the chloralose anaesthetized cat.

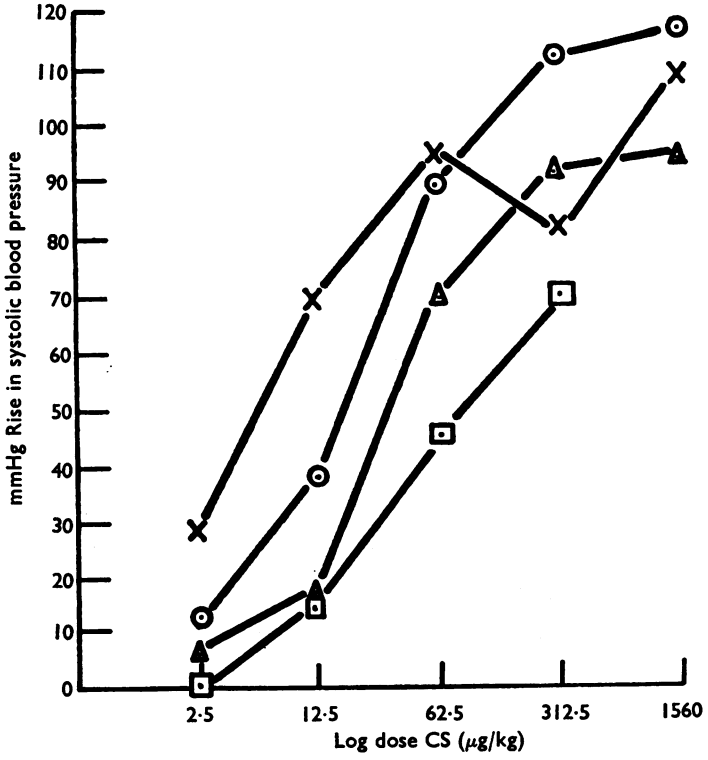

FIG. 3. Average rises in systolic blood pressure of the chloralose anaesthetized cat following the injection of CS by different intravascular routes. The routes of administration were carotid artery (×), aortic arch (○), right atrium (△) and femoral vein (□).

is illustrated graphically in Fig. 3. The minimal effective dose of CS was generally in the range 2·5 to 12·5 μg/kg although lower doses were often effective when given intraarterially into the carotid artery. Maximal effects occurred with doses of about 300 μg/kg.

In the chloralose anaesthetized dog the pattern of response varied somewhat with the route of administration of CS. This is illustrated in Fig. 4. By the femoral vein route, or when injected into the right atrium, no significant effects were seen with doses below 312·5 μg/kg. This dose produced a fall in blood pressure accompanied by a bradycardia, the effects being more marked after intra-atrial injection. There was a transient apnoea (about 12 s). When injected into the carotid artery CS had little effect at 12·5 μg/kg ; at 62·5 μg/kg there was a small biphasic response with blood pressure being first reduced and then increased ; at 312·5 μg/kg there was a characteristic biphasic response with a fall in blood pressure and bradycardia followed by an increase in blood pressure. The bradycardia persisted for some time after the blood pressure had returned to normal. This response was accompanied by a period of apnoea. Doses higher than 312·5 μg/kg given by the intravenous or intra-atrial routes produced very similar responses.

In rabbits anaesthetized with urethane no significant effects were observed with dose levels of CS below 62·5 μg/kg and this dose was only effective when given by the intracarotid route when it caused an apnoea of very short duration (about 2 s) followed by a small increase in respiratory rate and accompanied by a slight fall in blood pressure. An intracarotid dose of 312·5 μg/kg produced a brief apnoea (2–5 s) followed by a marked increase in respiratory rate (from 80–100 to 120–140 breaths per min). There was usually a fall in blood pressure of 20–40 mmHg. The same dose given intravenously had similar effects on respiration but little effect on blood pressure. Higher doses produced similar, but rather more marked, effects.

Studies in both cats and dogs showed that the pressor component of the response to CS was completely abolished by pretreatment with the α-adrenoceptor blocking drug phentolamine (2 mg/kg) and was antagonized to some extent by the ganglion-blocking drug, hexamethonium (5 mg/kg). Neither drug affected the respiratory response to CS.

In several cats the carotid sinus nerve was cut, or the carotid body was tied off, but these procedures had little or no effect on the responses to CS although they abolished responses to injected potassium cyanide. After vagotomy responses were either unaffected or slightly potentiated. In spinal cats the effects on blood pressure were abolished.

Some studies were made in which the type of anaesthetic used was varied. Cats were anaesthetized with pentobarbitone (20 mg/kg, i.p.) or with halothane. After

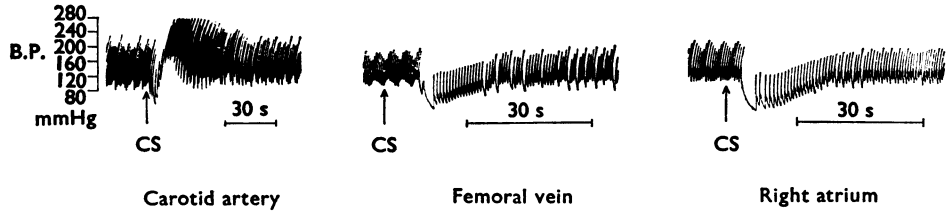

FIG. 4. Effects of CS (312·5 μg/kg), administered by different routes, on the arterial blood pressure of the chloralose anaesthetized dog.

pentobarbital anaesthesia the animals were less sensitive to the effects of CS with threshold doses being elevated by a factor of about 5 and maximum response levels being reduced as compared with those in the chloralosed cat. After halothane anaesthesia in both the cat and the rabbit doses of up to 1 mg/kg produced virtually no blood pressure changes but respiratory changes were little affected.

In some experiments very high intravenous doses of CS were given. The lethal dose was very variable but usually lay between 10 and 20 mg/kg. Death seemed to be due to cardiovascular collapse which preceded respiratory failure.

Three metabolites of CS were given to chloralosed cats by intravenous injection. They were *o*-chlorobenzaldehyde, 1-(*o*-chlorophenyl) 2(dinitrile) ethane (' dihydro-CS '), and malononitrile. None of these substances produced significant effects at doses up to and including 312·5 μg/kg. At 1·5 mg/kg the benzaldehyde and malononitrile produced small pressor responses (<20 mmHg) but still had no effect on respiration. Dihydro-CS at 1·5 mg/kg produced a depressor response and at

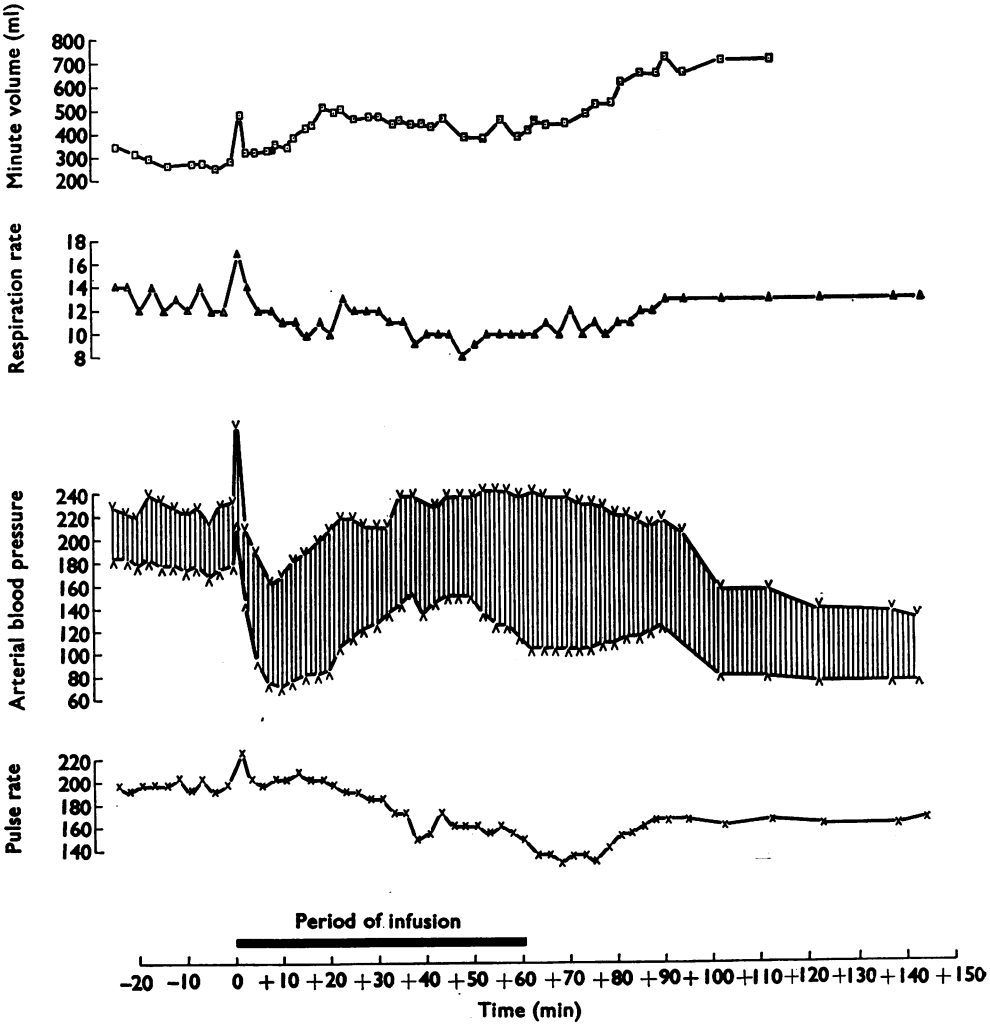

FIG. 5. Effects of CS solution ((0·33 mg/kg)/min), given by intravenous infusion for a period of 1 h, in the chloralose anaesthetized cat.

6·2 mg/kg a marked depressor response followed by an increase in blood pressure accompanied by a tachycardia. At this higher dose there was a transient apnoea followed by an increase in respiration rate.

Three cats were given CS solution by intravenous infusion. The total dose was 20 mg/kg given over 1 hour. A typical result is shown in Fig. 5. Each measured parameter was markedly modified almost immediately after the beginning of the infusion, respiratory rate and volume blood pressure and pulse rate were all increased, an effect almost identical to that seen after an intravenous injection of a large dose of CS. Subsequently the respiratory volume showed increases which were continued in the postinfusion period although there was some depression in rate. Blood pressure fell markedly but resumed the resting level after about 30 minutes. Pulse pressure was increased throughout the infusion period but began to fall, together with blood pressure, shortly after the end of the infusion period. Pulse rate progressively decreased during the infusion but there was some recovery afterwards.

Inhalation studies

Sixteen cats received pyrotechnically generated (grenade) CS via the upper respiratory tract; of these twelve were given high concentrations (Cts between (26,590 and 54,660 mg/min)/m^3) and four low concentrations (Cts between (3,650 and 4,310 mg/min)/m^3). Half of these cats had been pre-exposed to CS. Four cats received CS (Cts 34,370 to 62,630) via a tracheal cannula; three of these cats had been pre-exposed to CS. One cat received grenade combustion products but no CS via the upper respiratory tract and in one cat grenade CS was drawn through the upper respiratory tract but was prevented from entering the lungs. Three cats received pure CS, two via a tracheal cannula (Cts of 8,840 and 83,400) and one by the upper respiratory tract (Ct 21,258).

Examination of the results of these experiments revealed that very few consistent patterns of change occurred in any of the measured parameters. The changes which appeared to be of some significance were as follows:

(i) In all cats receiving grenade CS via the upper respiratory tract the time to onset of effect on respiration (apnoea or decrease in rate) lay between 34 and 44 s after ignition of the grenade when high concentrations were used, and between 50 and 60 s when lower concentrations were used. This seemed, in the main, to be due to the slightly different method used to produce lower concentrations which resulted in an additional delay between the time of ignition and the CS reaching the cat.

(ii) In all cats receiving grenade CS via the upper respiratory tract there was a very rapid decrease in respiratory rate, sometimes preceded by a short period of apnoea. The general trend was for the decrease in rate to be maintained for the period of the exposure to CS with subsequent partial recovery when the experiment terminated at 30 min after the end of exposure. In two cases where termination of the experiment was postponed recovery continued to the pre-exposure rate.

(iii) In all but two cats exposed via the upper respiratory tract to high concentrations of grenade CS there was an immediate, transient increase in blood pressure. This was not seen in cats exposed to lower concentrations. In all

cats there were small transient increases in pulse rate at the beginning of the period of exposure.

(iv) There were no significant differences between initial blood pO_2, pCO_2 or pH values in cats pre-exposed to grenade CS and those which had not been pre-exposed. The only consistent difference observed was that in the pre-exposed cats given CS by the upper respiratory route the mean fall in pO_2 over the first 10 min of exposure to grenade CS was significantly greater ($P<0{\cdot}02$) than those in non pre-exposed cats. After this first 10 min period there was a tendency either for pO_2 levels to increase when the animals survived or to continue to decrease when the animals died. Apart from a tendency to progressive elevation no marked patterns were apparent in the blood pCO_2 or hydrogen ion concentrations.

(v) Three out of six cats which had been pre-exposed to CS died during or after the acute exposure to a high concentration of CS; one out of six non pre-exposed cats died. Death appeared to be due to respiratory failure which preceded cardiovascular collapse. None of the four cats exposed to lower concentrations died irrespective of whether they had been pre-exposed or not.

(vi) In comparison with cats which received grenade CS via the upper respiratory tract those which received the material via a tracheal cannula showed a tendency towards an initial respiratory stimulation. This occurred in three out of four of the cats studied (2/3 pre-exposed, 1/1 non pre-exposed). In the other cat respiratory rate was unaffected. Apnoea was not seen in any cat; a degree of respiratory depression usually followed the stimulation. The time to onset of this respiratory stimulation was longer than the time to onset of the depression seen in cats given grenade CS via the upper respiratory tract. There were no immediate marked effects on blood pressure in these cats.

(vii) The non pre-exposed cats survived the exposure to high concentrations of grenade CS by tracheal cannula. One out of three pre-exposed cats died at about 30 min post-exposure.

(viii) Cats given pure CS by either route showed no marked or consistent effects.

(ix) No marked changes in any measured parameter were noted in the cat which received only grenade combustion products via the upper respiratory tract.

(x) In the cat in which the grenade CS was merely drawn through the upper respiratory tract there was apnoea, followed by a decrease in respiratory rate and an increase in blood pressure and heart rate.

Oral administration

Three cats were given CS by stomach tube. Doses of 10, 50 and 100 mg/kg were without effect on respiration, blood pressure or pulse rate.

Cat tibialis anterior preparation

One and 10 mg/kg CS produced a slight increase in twitch tension (8% and 16% respectively) accompanied by a 60 mmHg rise in blood pressure. Five minutes after injection of 10 mg/kg the tetanic response to a 50 Hz stimulus was similar to that

observed in the untreated preparation. The tetanus was well maintained and twitch tension recorded directly after the tetanus showed typical post-tetanic potentiation. Twenty milligrammes per kilogramme CS produced a 16% increase in twitch tension accompanied by a transient 60 mmHg fall in blood pressure and slowing of the respiration rate.

Cat encéphale isolé *preparation*

A dose of 10 μg/kg CS intravenously had no effect on either the behaviour or the electrocortical activity of this preparation. 100 μg/kg by the same route produced marked electrocortical (change from synchronized to desynchronized pattern) and behavioural (opening of the eyes and movements of the vibrissae) alerting. There was slight salivation. The duration of alerting was brief; sleep activity returned 3 min after drug administration. At this time there was no change in the thresholds for behavioural or electrocortical arousal evoked by high-frequency stimulation of the reticular formation. When given at 1 mg/kg CS produced marked electrocortical and behavioural alerting, salivation and a transient rise in blood pressure (5–30 mmHg). In no preparation did alerting persist for longer than 10 minutes. At that time sleep activity was present in the electrocorticogram and no changes in arousal thresholds were detected.

Ten milligrammes per kilogramme of CS produced a brief (about 30 s) period of alerting followed by severe cortical depression as shown by the disappearance of bursts of slow waves in the electrocorticogram. The cortical depression was accompanied by a marked fall in blood pressure. In two out of three preparations this dose level proved lethal. The electrocortical activity disappeared 3 min after injection and the blood pressure fell to zero. In the other preparation there was marked cortical depression for 4 min, accompanied by a 60 mmHg fall in blood pressure. By 20 min after injection the blood pressure and electrocortical activity had returned to normal. A further 10 mg/kg caused death within 3 minutes.

The effects of high doses of CS in producing cardiovascular collapse did not seem to be reduced by sectioning the vagi. In two vagotomized preparations there were rapid falls in blood pressure, one preparation dying after 10 mg/kg and the other after a total dose of 20 mg/kg.

Discussion

Any discussion of these results should be prefaced by stating that most of the routes used to administer CS and the doses given in these experiments are quite unrealistic in the context of its practical use. Additionally, the pure substance was used in many of the experiments rather than the products of any pyrotechnic devices although the latter were employed in some of the inhalation studies.

The results of the isolated guinea-pig ileum experiments indicate that CS had a weak stimulant action on the longitudinal muscle and an additional unspecific effect in that it depressed contractions elicited by both acetylcholine and histamine. The marked tachyphylaxis to repeated doses of CS and the waning nature of the contractor responses suggests that it may be stimulating rapidly adapting sensory receptors. Further evidence that the contractions are brought about by stimulation of nervous structures in the ileum is provided by the fact that they were blocked by both morphine and atropine. Atropine is a competitive antagonist to acetylcholine and morphine blocks its release from postganglionic nerve endings (Paton, 1957).

According to Beleslin (1969) compounds active in stimulating persistalsis should markedly restore peristalsis in the fatigued ileum. CS only elicited a short lasting restoration of peristalsis unlike carbachol which was very effective in this respect.

It seems unlikely, therefore, from these results, and particularly from the fact that CS given intraluminally had no effect on the peristaltic reflex, that it will facilitate peristalsis although very high doses may produce depression of the peristaltic reflex.

Doses of CS of up to 1 mg/kg produced no abnormalities in the pattern of electrocortical activity of cat *encéphale isolé* preparations. Behavioural and electrocortical alerting occurred but this effect was of short duration. Higher doses produced cortical depression associated with marked falls in blood pressure which seemed not to be due to any extent to reflex cardio-inhibition mediated through the vagus nerve since vagotomy modified the response very little.

Pressor effects seen in the *encéphale isolé* preparation were less marked than those in intact anaesthetized animals. This is presumably due to the fact that in the former preparation efferent sympathetic pathways had been sectioned.

The general conclusion to be drawn from the experiments in which CS was given by intravascular injection does not differ from that of Biscoe & Shephard (1962), that is, that the substance is acting as a non-specific irritant stimulating a variety of nervous reflexes. Many of the detailed differences between our findings and those of Biscoe & Shephard can be explained by the fact that the latter authors used ethanol as a solvent. This, as they acknowledge, exerted some pharmacological actions in its own right. The apparent higher potency of CS in our experiments was almost certainly due to the fact that chloralose, rather than a barbiturate, was used as the anaesthetic. In a few experiments where pentobarbitone was used threshold doses for CS were higher than in chloralose anaesthetized cats. The comparative lack of effect of the CS metabolites tested suggests that CS itself was responsible for the effects observed.

The commonly-observed pressor response was almost certainly due to a sympathetic discharge because the response was abolished by phentolamine. Hexamethonium and the anaesthetic halothane, also known to have ganglion-blocking properties, similarly blocked the pressor response.

Attempts to define the precise sites of action of CS were not successful. Biscoe & Shephard (1962) found that denervation of the carotid sinuses often reduced or abolished the pressor response but, on the other hand, they were unable to demonstrate increased neuronal discharge in afferent pathways from the sinus. We were unable to affect the response by denervation. No explanation can be offered for this especially as other evidence might have suggested involvement of the carotid body chemoreceptors in the response to CS. In particular, the fact that the material became progressively more potent as it was introduced nearer to the head and neck region seems to support this view.

Perhaps the most likely explanation is that central nervous structures, probably mainly medullary, are involved in the cardiovascular response to CS as shown by the fact that this response was absent in the spinal animal and reduced in the *encéphale isolé* preparation. The tendency for barbiturates to reduce the response was also probably due in part to their depressant actions on the medulla although these drugs also depress peripheral neural pathways.

Both qualitative and quantitative species differences were seen in these studies. For example, the dog seemed to be less sensitive than the cat to the cardiovascular effects of CS. In both the dog and the rabbit but not in the cat there was a regular tendency for marked respiratory stimulation to follow the period of apnoea. There are no explanations for these species differences at present.

The above effects of CS differ in some respects from those of the irritant substance capsaicin, the pharmacology of which was studied by Toh, Lee & Kiang (1955). Similar effects to those of CS were reported on isolated guinea-pig ileum but effects on the chloralosed cat were somewhat different. Apnoea occurred but this was abolished by cooling the vagi whereas in our experiments with CS vagotomy did not affect the period of apnoea. The other main difference was that capsaicin, especially when injected into the carotid sinus region, caused a fall in blood pressure. This we never observed with CS, there was always a pressor response.

Analysis of the results of the experiments in which CS was given by inhalation is made difficult by the complexity of factors involved, instrumental as well as pharmacological. Nevertheless, there was no evidence for any marked progressive changes in any of the measured parameters in control anaesthetized cats subjected to exactly the same procedures as CS-exposed cats, except that the agent was not given. Any effect noted in cats given pure CS by either the upper respiratory route or by tracheal cannula were not distinguishable from those seen in these control animals. However, when the same quantity of pure CS was given over the same period by intravenous infusion quite marked effects were seen. Initially, these resembled the effects of an acute injection but there were subsequent progressive changes with continued administration of the agent. These findings seem to demonstrate that the proportion of the inhaled dose of pure CS which reaches the blood stream is very small. In the experiment where CS was given by intravascular injection effects were regularly seen with doses of 2·5 μg/kg. In the inhalation experiment where a concentration of 1·39 g/m^3 was used the animal was inhaling about (316 μg/kg)/min but with no apparent effect.

In contrast with pure CS some effects on the physiological systems being monitored were noted when grenade CS was used. These effects differed according to the route of administration. Five out of sixteen of the cats receiving the material via the upper respiratory tract showed an initial period of apnoea of variable duration, none of the cats given CS by tracheal cannula displayed this effect. Most of the latter animals showed initial respiratory stimulation and unchanged or elevated blood pO_2 while all the former showed the reverse effect, that is, respiratory depression and depressed blood pO_2. This suggested a depressant reflex initiated in the upper respiratory tract and this was confirmed by the finding that respiratory depression occurred in a cat in which grenade CS was drawn through the upper respiratory tract but did not enter the lungs. In this cat an initial rise in blood pressure occurred similar to that observed in cats where CS was introduced into the lungs via the upper respiratory tract, again suggesting a reflex phenomenon.

Ignited standard pyrotechnic mixture had very little effect on the cats so at present there seems to be no satisfactory explanation for the rather more marked effects of grenade CS compared with pure CS.

It is very difficult from these results to say whether pre-exposure of cats to grenade CS had any influence on the subsequent response of the animals to high concentrations of CS. The lethality in the pre-exposed group given CS by the upper respira-

tory route was three out of six compared with one out of six in the group which was not pre-exposed. The corresponding figures in animals given CS by tracheal cannula were one out of three and nought out of one respectively, but in view of the small numbers the significance of this is doubtful. Certainly there were no differences in the blood gas analyses of the pre-exposed and control group before their final exposure under anaesthetic to a high concentration of CS. However, the pre-exposed group reacted to the CS more markedly in that falls in blood pO_2 were greater over the first 10 min of exposure than in non pre-exposed animals. No other differences between the two groups could be detected. In the four animals which were given lower concentrations of CS the responses of the two pre-exposed animals were indistinguishable in every respect from those of the animals not pre-exposed.

Extrapolation to man of results obtained using anaesthetized cats may not be completely justifiable but to put these experiments into context it is worth remarking that a concentration of CS of about 10 mg/m^3 is intolerable but even if such a concentration were inhaled for 1 h the total Ct would be (600 mg/min)/m^3. In certain conditions higher concentrations might be encountered. For example Ballantyne & Callaway (1972) report that if a L2A2 cartridge containing 12·5 g CS is functioned in a 20 m^3 room with low natural ventilation, that is one change per hour, the initial concentration of about 500 mg/m^3 will fall to about one-hundredth of that value in 30 minutes. Even if exposure is extended for more than 30 min the total dosage accumulated will not appreciably exceed (6,000 mg/min)/m^3. In our experiments where high concentrations of grenade CS were used they ranged from 400 to 1,040 mg/m^3 (Cts of (26,590 to 62,630 mg/min)/m^3) and in the experiments where lower concentrations were used they ranged from about 60 to 70 mg/m^3 (Cts of (3,650 to 4,310 mg/min)/m^3). Animals exposed to these lower concentrations showed certain effects discussed above but these did not progress after exposure was terminated and when animals were kept for more than 30 min after the exposure period there was a tendency for recovery in all measured parameters.

An additional point is that great increases in Ct were not followed by proportionate increase in effects on the respiratory or cardiovascular systems.

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