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STUDIES ON THE PHARMACOLOGY OF CONOPHARYNGINE, AN INDOLE ALKALOID OF THE VOACANGA SERIES

BY

P. R. CARROLL AND G. A. STARMER

From the Department of Pharmacology, University of Sydney, New South Wales, Australia

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Conopharyngine, the major alkaloid present in the leaves of *Tabernaemontana* (*Conopharyngia*) *pachysiphon* var. *cumminsi* (Stapf) H. Huber was isolated and identified by Thomas & Starmer (1963). The same alkaloid has also been found in the stem bark of a Nigerian variety of the same species by Patel & Poisson (1966) and in the stem bark of *Conopharyngia durissima* by Renner, Prins & Stoll (1959). Conopharyngine is an indole alkaloid of the voacanga type, being 18-carbomethoxy-12,13-dimethoxyibogamine (Fig. 1) and is thus closely related to voacangine and coronaridine.

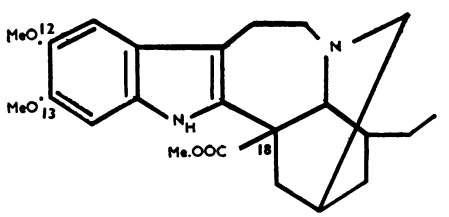

Fig. 1. Conopharyngine (18-carbomethoxy-12,13-dimethoxyibogamine).

Some confusion exists in that an alkaloid with an entirely different structure, but also named conopharyngine, was isolated from a cultivated variety of *Conopharyngia pachysiphon* by Dickel, Lucas & Macphillamy (1959). This compound was shown to be the 3-D-β-glucoside of Δ^5-20α-amino-3 β-hydroxypregnene, and was reported to possess marked hypotensive properties. The presence of steroid alkaloids in the Tabernaemontaneae was hitherto unknown and it was suggested by Raffauf & Flagler (1960) and Bisset (1961) that the plant material was open to further botanical confirmation.

The roots of the conopharyngia species are used in West Africa to treat fever (Kennedy, 1936), including that of malaria (Watt & Breyer-Brandwijk, 1962).

The only report on the pharmacology of conopharyngine is that of Zetler (1964), who included it in a study of some of the effects of 23 natural and semi-synthetic alkaloids

from the Tabernaemontaneae. Conopharyngine was shown to possess central nervous stimulant activity and to produce bradycardia and hypotension in the anaesthetized cat.

This paper presents the results of a more detailed study of the pharmacology of conopharyngine. Comparisons are drawn with the reported effects of closely related alkaloids.

METHODS

Animals

All animals used in these investigations were obtained from the Sydney University animal farm. Mice were albinos of the QS-strain weighing 18–22 g. Rats were of the Wistar strain and the rabbits New Zealand whites. Large guinea-pigs (400 g) were used for the smooth muscle experiments.

Behavioural changes were investigated in the mouse, the rat and the cat. The animals were observed continuously for 4 hr after administration of conopharyngine and at intervals for 2 days. Overt changes were recorded.

Acute toxicity. Conopharyngine was administered intravenously to groups of 10 male mice. Deaths were counted after 48 hr and the LD50 and its limits of error were calculated by the method of Litchfield & Wilcoxon (1949).

Co-ordinated locomotor activity. The effect of conopharyngine on the exploratory activity of mice released into a new environment was investigated by a method based on that of Dews (1953). Groups of 10 male mice were used at each dose level and the drug was given by intraperitoneal injection. Readings of the photoelectric counter were taken every 5 min for 1 hr and cumulative plots of activity compared with that for a saline control.

Effects on hexobarbitone anaesthesia were investigated by the method of Holten & Larsen (1956). Ten male mice were used at each dose level. An ambient temperature of 25° C was maintained throughout the experiment and the animals received the drug or a saline control intraperitoneally 30 min before the hexobarbitone was administered.

Leptazol antagonism by conopharyngine was investigated using the method of Goodman, Grewal, Brown & Swinyard (1953). The mice were injected intraperitoneally with the alkaloid 30 min before the leptazol was given. The protection of the mice against lethal doses of leptazol was used as a measure of the activity of conopharyngine.

Effect on body temperature. Male rabbits (1.5–2.5 kg), which had been deprived of food overnight, were placed in individual boxes and restrained in neck stocks. Rectal thermocouples were inserted to a depth of about 7 cm and galvanometer readings taken half-hourly for 6 hr. After the third reading the animals received the drug or a saline control intraperitoneally.

Analgesia. Modifications of the hotplate method (Woolfe & Macdonald, 1944) and the writhing test (Hendershot & Forsaith, 1959) were used to investigate the analgesic effects of conopharyngine in mice.

The surface temperature of the hotplate was maintained at $55 \pm 0.5°$ C and flicking of the hind paws was used as an index of pain. A quantal assessment of analgesia was made, the critical reaction time being calculated from the initial reaction times of the animals (mean initial reaction time +2 standard deviations). Twenty mice were used at each dose level and the drug was given intravenously. The mice were placed on the hotplate every 30 min for 3 hr. A saline control group, subjected to this procedure, gave a positive reaction to the noxious stimulus on each occasion. A test for loss of co-ordination (Collier, Hall & Fieller, 1949) was carried out on these animals each hour during the experiment. The influence of nalorphine on analgesia induced by conopharyngine at peak effect was investigated.

The conditions of the writhing test were essentially those of Parkes & Pickens (1965) except that formic acid (25 mg/kg) was used as the writhing agent. Conopharyngine was injected subcutaneously 30 min before the formic acid and 12 mice were used at each dose level. The animals were placed

in individual observation chambers and the number of writhing episodes which occurred in each animal during the first 20 min after injection of the irritant were counted.

Local anaesthetic action was investigated in the rabbit after conjunctival instillation (Sollman, 1918) and in the mouse by estimation of the inhibition of the response to pressure in the mouse tail after local subcutaneous injection of conopharyngine. Ten mice were used at each dose level and pressure was applied over the injection site by means of a small artery clip every 10 min for 1 hr. Animals which did not attempt to remove the clip within 30 sec were considered to be anaesthetized.

Isolated tissue experiments. Experiments on isolated smooth muscle preparations were performed at 30–32° C using a 20 ml. organ bath and 95% O_2:5% CO_2 aeration. Contractions were recorded on a smoked drum by means of an isotonic lever. Rat uteri were from animals given stilboestrol (0.02 mg intramuscularly) 36 hr previously. The isolated vas deferens-hypogastric nerve preparation of the guinea-pig was set up as described by Huković (1961). The contractions of isolated guinea-pig and rabbit atria were recorded by means of a Starling Heart Lever (C. F. Palmer, Ltd., London). A Perspex organ bath of rectangular cross section was used for the rat diaphragm-phrenic nerve preparation. This experiment was performed at 25° C and the phrenic nerve was stimulated by square wave pulses (10–20V, 0.1–1.0 msec) delivered at a rate of 6/min. Contractions of the diaphragm were recorded using a Brodie universal lever (C. F. Palmer, Ltd., London). The formulae of the physiological solutions used in these experiments are listed in Table 1.

TABLE 1

COMPOSITION OF PHYSIOLOGICAL SOLUTIONS USED IN ISOLATED TISSUE EXPERIMENTS (g/l.)

	Rabbit duodenum	Guinea-pig ileum	Rat uterus	Guinea-pig vas deferens	Rat diaphragm-phrenic nerve	Rabbit and guinea-pig atria
NaCl	8·0	8·0	9·0	6·6	8·02	6·92
KCl	2·0	2·0	0·42	0·35	0·2	0·35
$CaCl_2.2H_2O$	0·65	0·86	0·29	0·28	0·2	0·37
$MgSO_4.7H_2O$	—	0·29	—	0·294	0·025	0·29
NaH_2PO_4	0·65	—	—	—	0·058	—
KH_2PO_4	—	0·16	—	0·162	—	0·165
$NaHCO_3$	1·1	1·1	0·5	2·1	1·0	1·8
Dextrose	1·0	1·0	0·5	2·08	2·0	2·1

Effects on gastro-intestinal motility were investigated in mice using the method of Macht & Barba-Gose (1931). The animals were fasted overnight and received the drug or a saline control intraperitoneally 30 min before the charcoal suspension. The mice were killed after 30 min and the distance travelled by the charcoal along the small intestine was measured.

Neuromuscular blocking activity was investigated in the mouse. The animals received conopharyngine intraperitoneally, three mice being used at each dose level. The ability of the animals to retain their grip on a slowly revolving wire mesh drum (9 in. diam, 2 rev/min) was observed for 60 min after administration of the drug.

Experiments on anaesthetized cats. Cats were anaesthetized with chloralose (50 mg/kg, intraperitoneally). The blood pressure was recorded manometrically after carotid cannulation. Drugs were injected into the femoral vein. Contractions of the nictitating membrane were recorded on the smoked drum by means of an isometric lever. The superior cervical ganglion was exposed and preganglionic stimulation by square wave pulses (5V, 1 msec duration, 2/sec) was administered for 15 sec to obtain submaximal contractions. In some experiments conopharyngine was applied directly to the ganglion.

Drugs

Conopharyngine was dissolved in 0.1N HCl and the pH was adjusted to between 5 and 5.5 with 0.1N NaOH. Concentrations of conopharyngine refer to the base. All other drugs were of B.P. or analytical grade.

RESULTS

Behavioural changes

In mice, conopharyngine (50–100 mg/kg, intravenously) produced marked vasodilatation of the ears and paws which lasted for several hours, piloerection, salivation and diarrhoea. Intermittent periods of increased and decreased activity were observed and the animals exhibited a coarse tremor. Higher doses (above 100 mg/kg intravenously) caused immediate ataxia and dyspnoea followed by tonic-clonic convulsions and opisthotonos. A dose of 200 mg/kg intravenously was lethal within 2.5 min.

The rat was more sensitive to the drug ; 60 mg/kg intraperitoneally caused a flaccid paralysis of the voluntary muscles and death within 1 min. At lower dose levels (15–30 mg/kg, intraperitoneally) vasodilatation, piloerection, tremor, and head shaking were observed. The animals failed to react to a mechanically induced pain stimulus to which they were previously sensitive. No overt effects were seen in the cat below a dose level of 60 mg/kg intraperitoneally where a slight loss of co-ordination, pupillary dilatation and an insensitivity to both thermally and mechanically induced pain were observed.

Acute toxicity

The intravenous LD50 of conopharyngine in mice was found to be 143 mg/kg with limits of error 126–162 for $P=0.05$.

Co-ordinated locomotor activity

Conopharyngine was seen to modify the exploratory activity of mice. Small doses (5–20 mg/kg, intraperitoneally) caused a reduction of activity below the control level, whereas higher doses (40–80 mg/kg) increased activity. Above 120 mg/kg activity was greatly reduced and the acute toxic effects of the drug were exhibited.

Effect on body temperature

Conopharyngine given intraperitoneally at 10, 50 and 100 mg/kg, had no significant effect on the body temperature of rabbits.

Effects on hexobarbitone anaesthesia

Administration of hexobarbitone alone (100 mg/kg, intraperitoneally) induced anaesthesia which lasted for 31.6 ± 3.1 min (S.E. of mean). Pretreatment with conopharyngine (50 mg/kg) 30 min before the hexobarbitone increased the sleeping time to 95.1 ± 4.3 min ($P<0.001$). This increase was very highly significant.

Leptazol antagonism

Conopharyngine (50 mg/kg, intraperitoneally), given 30 min before leptazol (60, 80, 100 and 120 mg/kg, intraperitoneally) had no effect on the mortality rate. At 100 mg/kg slight antagonism was seen, manifested by an increase in survival time.

Analgesic effects

Calculation of the results on the hotplate method (Litchfield & Wilcoxon, 1949) gave an ED50 of 26 mg/kg with limits of error of 17–41 ($P=0.05$). Loss of co-ordination was

seen only above 80 mg/kg, at which dose level 20% of the animals were unable to retain their grip on the drum for the requisite time. The analgesic effects of conopharyngine were not reversed by nalorphine (5–10 mg/kg, intravenously).

The slope and position of the regression line in the writhing test was calculated by standard statistical methods (Burn, Finney & Goodwin, 1950) and from this the ED50 and its limits of error. This was found to be 6 mg/kg (limits of error 4.9–6.7 for $P = 0.05$).

Local anaesthesia

On the rabbit eye, instillation of a 5 mg/ml. solution of conopharyngine into the conjunctival sac did not produce local anaesthesia. The pH of this solution was 5.6 and more concentrated solutions of the drug were too irritant for application to the eye. A 1 mg/ml. solution at pH 6.3 had no local anaesthetic action.

In the presence of adrenaline 10^{-6} g/ml., 1 mg of conopharyngine injected subcutaneously into the mouse tail produced local anaesthesia after 20 min in all the animals tested while 0.5 mg produced local anaesthesia in four out of 10 mice. Procaine, tested under the same experimental conditions was approximately twice as potent.

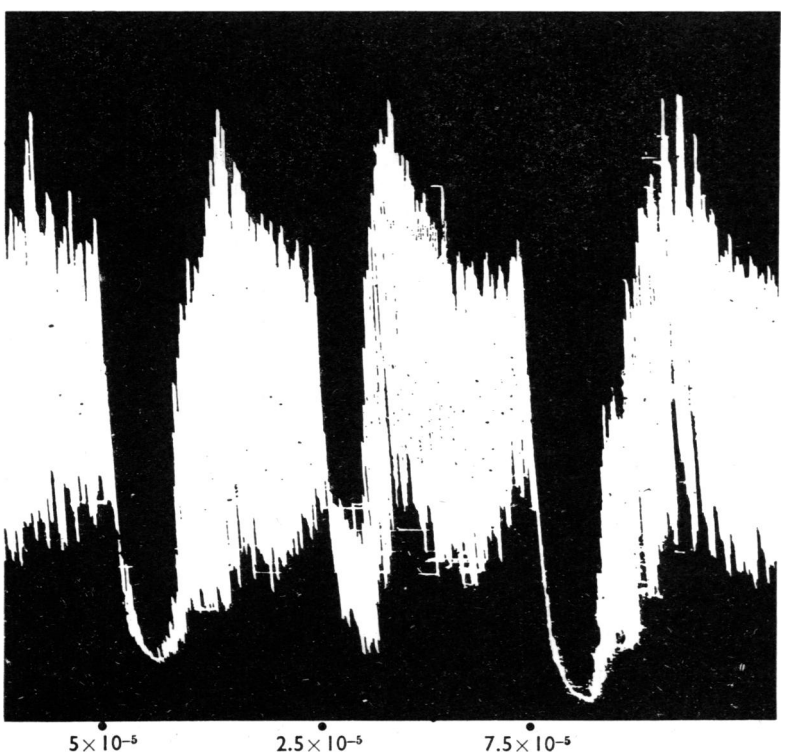

Fig. 2. The rabbit duodenum. The effects of conopharyngine (●) on the tone and motility of the preparation. Doses expressed in g/ml.

Rabbit duodenum

Conopharyngine inhibited the spontaneous movement of the preparation and lowered its tone (Fig. 2). The threshold for this effect was between 5×10^{-6} and 10^{-5} g/ml. and the response was dose dependent, a tenfold increase giving maximum relaxation. Conopharyngine was found to be 2.5–3.5 times less potent than papaverine in reducing the tone of the preparation.

Guinea-pig ileum

Antagonism of a wide variety of stimulant drugs was produced by conopharyngine. These included acetylcholine, histamine, nicotine, bradykinin, 5-hydroxytryptamine, potassium chloride, barium chloride, tetramethylammonium and carbachol. Thresholds for antagonism were between 5×10^{-5} and 10^{-6} g/ml., except against nicotine and tetramethylammonium where the threshold was in the region of 5×10^{-7} g/ml. Parallel log dose-response lines were plotted for the antagonism of all these agents (Fig. 3). Papaverine was 2.5–3.5 times as potent as conopharyngine in antagonizing the effects of acetylcholine, histamine and bradykinin but equipotent against nicotine. The log dose-response lines of conopharyngine and papaverine for antagonism of all the stimulants tested were found to be parallel.

Rat uterus

Conopharyngine antagonized the stimulant effects of oxytocin and bradykinin on this preparation with a threshold concentration of 10^{-6} g/ml.

Guinea-pig vas deferens

The contraction of the vas deferens after stimulation of the hypogastric nerve was inhibited by conopharyngine. This effect was dose dependent (Fig. 4).

Rat diaphragm-phrenic nerve preparation

At a high concentration (10^{-4} g/ml.) conopharyngine induced complete neuromuscular block within 5 min, the muscle remaining responsive to direct stimulation. Neostigmine and physostigmine had no effect on the block but a transient antagonism occurred after potassium chloride (1.6 mg/ml.).

Isolated auricle preparations of guinea-pig and rabbit

Negative inotropic and chronotropic effects were observed on these preparations with a bath concentration of 10^{-4} g/ml. conopharyngine. The rate was reduced by approximately one-third and the force of contraction by one-half. These effects were not antagonized by atropine.

Effects on gastro-intestinal motility

In the control animals the charcoal meal travelled 47.1 ± 3.4 cm (S.E. of mean) along the small intestine. Conopharyngine, given intraperitoneally in doses of up to 50 mg/kg, produced no significant effects but after 100 mg/kg a retardation of the passage of the meal occurred (16.1 ± 4.5 cm [S.E. of mean]). This retardation was very highly significant ($P<0.001$).

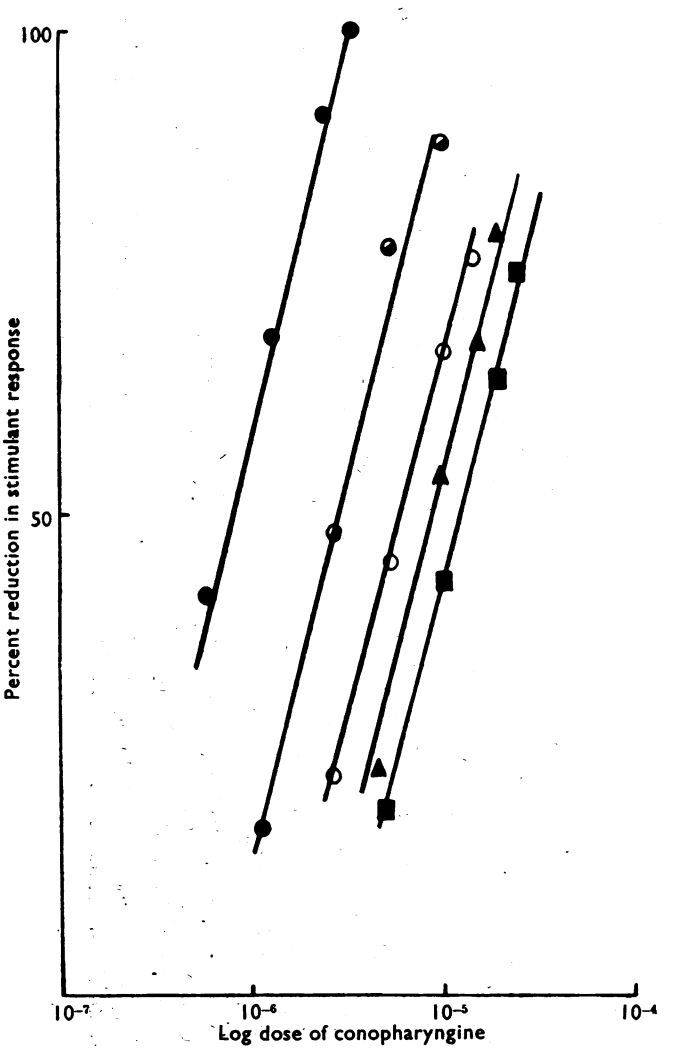

Fig. 3. Log dose-response curves for conopharyngine antagonism obtained against nicotine (●), tetramethylammonium (◑), histamine (○), carbachol (▲) and acetylcholine (■) on the guinea-pig ileum. Doses in g/ml.

Neuromuscular blocking activity in the mouse

The ability of mice to retain their grip on the rolling drum was not impaired by conopharyngine (20–120 mg/kg, intraperitoneally).

Experiments on the anaesthetized cat

Conopharyngine produced hypotension and bradycardia in the anaesthetized cat. The fall in blood pressure was relatively brief, dose dependent, and was not abolished by

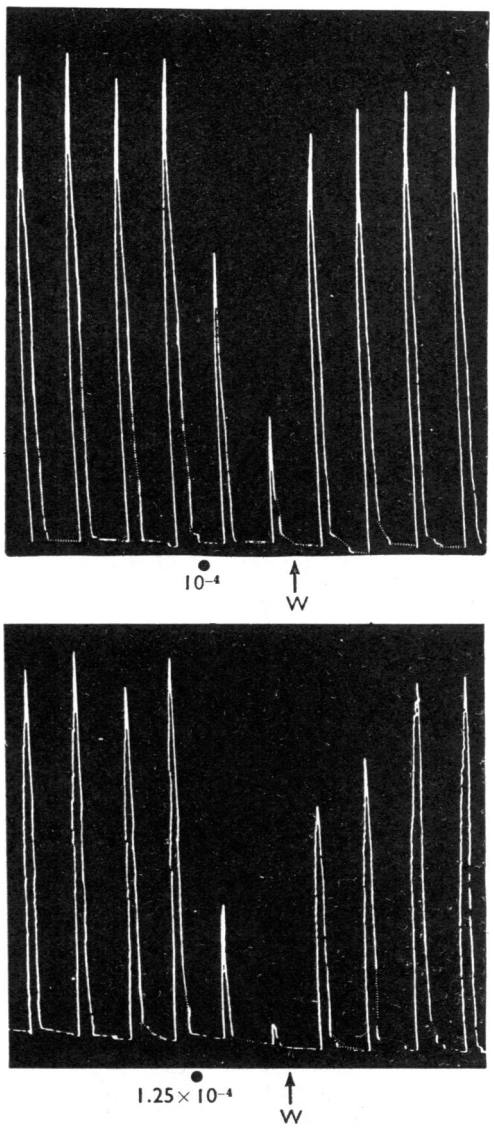

Fig. 4. The guinea-pig vas deferens. The effect of conopharyngine (●) on the contractions produced by electrical stimulation of the hypogastric nerve. Doses in g/ml. W=wash.

atropine (Fig. 5) or vagotomy. A second fall in blood pressure occurred in some animals about 30 min after injection of the alkaloid ; this response was less profound but lasted much longer (40 min) than the first fall.

A 5 mg/ml. solution of conopharyngine applied directly to the superior cervical ganglion abolished the response of the nictitating membrane to preganglionic stimulation. The response returned to normal after repeated washing with 0.9% sodium chloride solution. Intravenous injection of conopharyngine (10 mg/kg) was almost without effect on the response of the nictitating membrane.

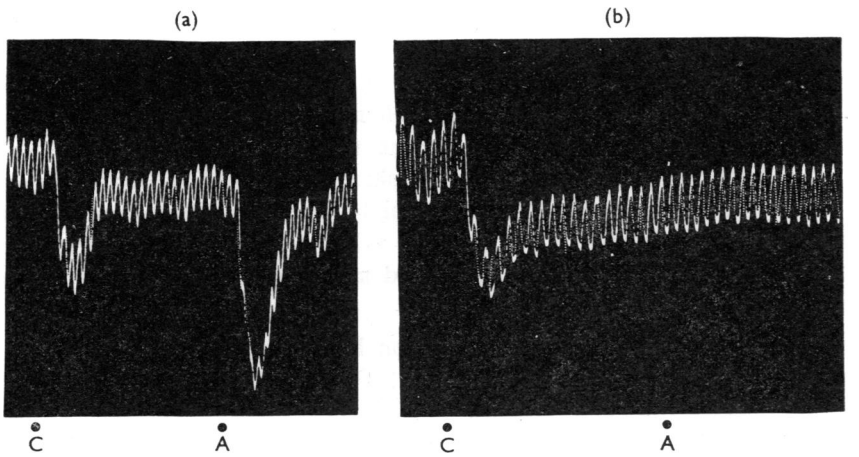

Fig. 5. The cat blood pressure. (a) The effect of acetylcholine (A) 1.5×10^{-5} g/kg and conopharyngine (C) 5×10^{-3} g/kg. (b) The effect of the same compounds in the presence of atropine (2.5×10^{-3} g/kg).

DISCUSSION

Conopharyngine is a member of a group of closely related alkaloids which differ only in the number and location of methoxy groups at C_{12} and C_{13} and the presence (voacanga series) or absence (iboga series) of a carbomethoxy group at C_{18}. The nature and positions of these substituents are given in Table 2. It was considered reasonable to compare the pharmacological actions of conopharyngine with those of the other members of the voacanga series and with ibogaline.

TABLE 2

THE STRUCTURAL RELATIONSHIPS OF SOME INDOLE ALKALOIDS OF THE IBOGA AND VOACANGA SERIES

Substituent at		Voacanga series	Iboga series
C_{12}	C_{13}	$COOCH_3$ at C_{18}	H at C_{18}
H	H	Coronaridine	Ibogamine
CH_3O	H	Voacangine	Ibogaine
H	CH_3O	Isovoacangine	Tabernanthine
CH_3O	CH_3O	Conopharyngine	Ibogaline

The overt effects of conopharyngine in the mouse were found to be similar to those described by Zetler (1964) except that catalepsy was not observed. The hypersensitivity of the rat found in these experiments is in contrast to that observed in the cat for coronaridine (Kupchan, Bright & Macko, 1963). Although mydriasis occurred in the cat there were no signs of the excitement, fear and rage reported after coronaridine (Kupchan *et al.*, 1963).

The acute intravenous toxicity of conopharyngine in the mouse is much lower than that of any of its close congeners, our findings being in complete agreement with those of Zetler (1964).

A fivefold increase in the voluntary activity of mice after conopharyngine (30 mg/kg, intraperitoneally) was reported by Zetler (1964) and a similar but less marked effect after coronaridine, voacangine and isovoacangine. Others workers (Blanpin, Quevauviller & Pontus, 1961 ; Vogel & Uebel, 1961) with different techniques, found that voacangine decreased voluntary activity. Using the same type of method as Zetler we did not observe any such clear-cut effects after conopharyngine. Activity was decreased after small doses of the alkaloid and increased by higher doses. In both cases the pattern of exploratory activity differed from that of the saline controls and was characterized by intermittent periods of increased and decreased movement similar to that reported after coronaridine (Kupchan *et al.*, 1963).

The potentiating effect of conopharyngine on hexobarbitone anaesthesia is similar to that reported for voacangine (Zetler & Unna, 1959 ; Vogel & Uebel, 1961 ; Blanpin *et al.*, 1961) although coronaridine was found to be inactive in this respect (Kupchan *et al.*, 1963).

Conopharyngine gave virtually no protection against the convulsant effects of leptazol, thus resembling coronaridine (Kupchan *et al.*, 1963) but differing from voacangine which was found (Zetler & Unna, 1959 ; Vogel & Uebel, 1961) both to retard the appearance of convulsions and to reduce their severity. Blanpin *et al.* (1961) were unable to show a protective effect with voacangine but reported a retardation, which we also observed with conopharyngine at high dose levels.

In the rabbit, conopharyngine, even at high dose levels, had no significant effects on body temperature in spite of the fact that vasodilatation was evident. Voacangine was found to lower body temperature in the mouse (Zetler & Unna, 1959) and the rabbit (Blanpin *et al.*, 1961) and to produce blockade of the thermoregulator system in the rat (Vogel & Uebel, 1961).

The analgesic effects of conopharyngine in the mouse were striking at dose levels where loss of co-ordination was insignificant. Tested under the same conditions the ED50 for morphine was found to be 5.2 ± 0.6 mg/kg (S.E. of mean) by the hotplate method and 1.5 ± 0.2 mg/kg (S.E. of mean) by the writhing test. Coronaridine has been shown to possess considerable analgesic potency (Kupchan *et al.*, 1963), but voacangine had neither analgesic activity nor potentiated the effects of methadone (Vogel & Uebel, 1961). Blanpin *et al.* (1961), however, did find weak activity for voacangine in the mouse against both thermally and mechanically induced pain.

The local anaesthetic activity of conopharyngine is not as marked as that reported for voacangine (Blanpin *et al.*, 1961). The lack of demonstrable activity on the rabbit cornea was possibly due to the lack of non-ionized molecules of the acidic conopharyngine hydrochloride solution (pH 5.6).

The inhibitory effects of conopharyngine on the gastrointestinal tract *in vivo* were consistent with its actions on isolated smooth muscle preparations. This is in contrast with the effects of voacangine (Vogel & Uebel, 1961 ; Blanpin *et al.*, 1961), which was stimulatory *in vivo* and inhibitory *in vitro*.

On isolated smooth muscle preparations conopharyngine exhibited rather general relaxant properties. The greater specificity of conopharyngine in antagonizing the effects of ganglion stimulants can be explained in terms of local anaesthetic rather than ganglion

blocking activity. Vogel & Uebel (1961) found that voacangine had spasmolytic effects on the isolated guinea-pig ileum against barium chloride but not against histamine and carbachol, whereas Blanpin *et al.* (1961) reported antagonism to the stimulant effects of acetylcholine, histamine and nicotine. We examined the effects of voacangine on the guinea-pig ileum against carbachol, barium chloride and histamine and found its actions to be essentially similar to those of conopharyngine, both qualitatively and quantitatively.

The iboga alkaloids have been shown to possess anticholinesterase activity (Raymond-Hamet & Vincent, 1960) but conopharyngine (Thomas, personal communication, 1966) was found to have only weak activity against acetylcholinesterase. A 22% inhibition of acetylcholinesterase was obtained at a concentration of 7.9×10^{-4} molar at pH 6.5 and 37° C. The lack of solubility of the alkaloid at pH 6.5 prevented determination of an I_{50}.

Neuromuscular blockade was produced in the rat diaphragm-phrenic nerve preparation only by high concentrations of conopharyngine and could not be shown in the mouse by the rolling drum method. The flaccid paralysis of the voluntary muscles which occurred in the rat at low dose levels could explain the hypersensitivity of this species to the drug.

The alkaloids of this series have often been reported to be cardiotonic (Janot & Goutarel, 1955 ; La Barre & Gillo, 1955). The negative inotropic and chronotropic effects exerted by conopharyngine on isolated atria are similar to those reported for voacangine (Blanpin *et al.*, 1961).

Hypotension and bradycardia were produced by conopharyngine in the anaesthetized cat. They were not abolished by atropine or vagotomy. Like Zetler (1964), we also observed a two-stage hypotensive effect in some animals.

Hypotensive effects have been demonstrated for all the alkaloids listed in Table 2 by Zetler (1964) in the anaesthetized animal but it is interesting to note that voacangine has been reported to produce hypertension in the conscious dog (Blanpin *et al.*, 1961).

The blockade of the response of the nictitating membrane to electrical stimulation after the direct application of conopharyngine to the superior cervical ganglion is largely a function of its local anaesthetic activity. Weak ganglion blocking activity has been reported for conopharyngine and ibogaline (Zetler, 1964), but not for voacangine (Blanpin *et al.*, 1961).

Thus it can be seen that qualitative as well as quantitative differences exist between the pharmacological actions of conopharyngine and those of voacangine and coronaridine. Most obvious is the relatively high analgesic activity of conopharyngine, yet it is difficult to appreciate from structure-activity considerations how the addition of one methoxy group to coronaridine will abolish analgesic activity and the addition of two such groups will greatly increase it.

SUMMARY

1. The pharmacological actions of conopharyngine have been investigated and comparisons drawn with the reported effects of closely related alkaloids.

2. Conopharyngine has been shown to possess potent analgesic activity unaccompanied by a loss of co-ordination. This action was not morphine-like.

3. On smooth muscle preparations conopharyngine exerted general relaxant effects.

4. Negative inotropic and chronotropic effects were observed on the isolated heart, and on the anaesthetized cat hypotension and bradycardia were produced which were not abolished by atropinization or vagotomy.

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