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Pharmacological studies of a new antitussive, 4-phenyl-l-piperidinecarboxamide (AH 1932)

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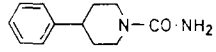
4-Phenyl-1-piperidinecarboxamide (AH 1932) markedly inhibits coughing induced in laboratory animals by chemical or mechanical irritation of the respiratory tract or by electrical stimulation of the superior laryngeal nerve. The compound is effective administered orally or parenterally and is at least as active as codeine. In contrast to codeine the antitussive activity of AH 1932 persists for 6 hr after oral administration. The evidence suggests a central site of action for AH 1932. The drug has a low acute toxicity in mice and rats, is devoid of analgesic activity, has no effect on the respiratory system and does not affect gastrointestinal propulsion. Cardiovascular effects are minimal. AH 1932 possesses weak spinal interneuron blocking activity unlikely to limit its use as a cough suppressant.

ADEQUATE doses of narcotic antitussive agents are effective in supressing cough but their use is accompanied by undesirable side-effects such as tolerance, addiction, respiratory depression, nausea and constipation. These limitations emphasize the need for new effective agents having selective antitussive properties. In animal tests 4-phenyl-l-piperidinecarboxamide (AH 1932) appears to be such a compound.



AH 1932

Experimental

ACUTE TOXICITY

Acute toxicity was determined following oral administration in male albino mice, Glaxo A_2G strain, weighing 18–22 g and male albino Wistar rats weighing 110–130 g. Deaths were recorded at 7 days. LD 50 values were calculated by the method of Litchfield & Wilcoxon (1949).

EFFECTS ON NORMAL BEHAVIOUR IN THE MOUSE, RAT, RABBIT, CAT AND DOG

The test compound was administered orally at different dose levels to each species as shown in Table 1 and the onset, character and intensity of drug effects were observed. In the mouse and rat, in addition to visual assessment of drug effects, the animals were handled to obtain information about their muscle tone, coordination and reflexes by a method similar to that described by Irwin (1963). The animals were then left undisturbed to recover from the effects of these manipulations. The process was repeated at intervals to assess the duration of drug activity and the time at which peak effects occurred. In the rabbit, cat and dog only gross changes in behaviour following drug administration were recorded.

ANTITUSSIVE ACTIVITY

Antitussive activity was determined against cough induced by chemical or mechanical irritation of the respiratory tract in conscious guinea-pigs or anaesthetized cats respectively and also by electrical stimulation of the superior laryngeal nerve in anaesthetized cats.

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TABLE 1. SPECIES, STRAIN, SEX, WEIGHT RANGES AND NUMBER OF ANIMALS USED IN BEHAVIOURAL STUDIES

Species	Strain	Sex	Body weight range	No. animals per dose level
Albino mouse	A_2G (Glaxo)	Male	18–22 g	3
Albino rat	Wistar	Male	110–130 g	3
Rabbit	Dutch	Male & Female	1·8–2·6 kg	1 Male, 1 Female
Cat	—	Male & Female	2·0–4·2 kg	1 Male, 1 Female
Dog	Mongrel	Male & Female	8·0–13 kg	1 Male, 1 Female

Guinea-pig. Experimental coughing was induced by inhalation of ammonia vapour (Winter & Flataker, 1954). Male albino guinea-pigs weighing 250–450 g were placed individually in a Perspex chamber through which air was passed either directly or after bubbling through 20% aqueous ammonia. A rotameter was included in the system to ensure constant air flow and an air escape prevented build up of pressure. A recording tambour was joined to the chamber to record changes in pressure due to coughing. Guinea-pigs were exposed to ammonia vapour for 90 sec and after this time air was passed directly into the chamber for a further 90 sec. The number of coughs during the 3 min period was determined. [The record of each cough is characteristic, is easily distinguishable from responses produced by sneezing, deep expirations, locomotor or grooming movements.] On the following day the procedure was repeated after animals had received test or reference compounds orally 90 min before exposure to the amonia vapour. The chamber was well ventilated between individual tests and the ammonia solution was changed after 6 experimental test periods. Five or 6 animals were used in each group. Antitussive activity was expressed as the percentage inhibition of coughing, each animal serving as its own control. While a high variation existed between animals in their sensitivity to ammonia, the responses of individual guinea-pigs did not vary by more than 5% on repeated exposure to ammonia. ED 50 values were calculated from the regression line of percentage inhibition plotted against dose.

Antitussive activity was also determined in guinea-pigs against coughing induced by sulphur dioxide. The method used was essentially that described by Miller, Robbins & Meyers (1963).

Cat. Male and female cats weighing 2·8–4·5 kg were anaesthetized with pentobarbitone sodium, 45 mg/kg intraperitoneally. Coughing was induced either mechanically by passing a polythene tube down the trachea until it touched the carina and then withdrawing it immediately (May & Widdicombe, 1954), or by electrical stimulation of the superior laryngeal nerve (Domenjoz, 1952). In the latter method the central end of the ligated nerve was stimulated for 5–15 sec with a train of rectangular pulses of 1–10V strength and 10–15 msec width, at a frequency of 5–10 impulses/sec. The interval between successive stimuli was 5 min in both techniques Diaphragmatic movements were recorded on smoked paper by an isotonic lever attached by a thread to the skin over the xiphisternum. Inhibition of the coughing was assessed by measuring the height of the records obtained, and expressed as a percentage of the control height.

ANALGESIC ACTIVITY

Groups of 10 mice received the test compound orally. One hr after drug administration, analgesic activity was investigated by determining the ability of a compound to inhibit writhing induced by phenylquinone (Brittain, Lehrer & Spencer, 1963) and to inhibit nociceptive response in a standardized tail-pinch method (Bianchi & Franceschini, 1954).

ANTICONVULSANT ACTIVITY

Anti-leptazol test. Groups of 10 mice received the test compound orally 90 min before a subcutaneous injection of leptazol, 100 mg/kg. The number of mice protected against the convulsive and lethal effects of leptazol was recorded.

Anti-maximal electroshock test. The test compound was administered orally to groups of 10 mice. Electric shock was applied through ear electrodes 2 hr after drug administration (Cashin & Jackson, 1962), and the number of animals showing no tonic extension of the hind limbs was recorded.

NEUROLEPTIC ACTIVITY

The method was based on the antagonism of amphetamine-induced toxicity in mice housed under crowded conditions (Burn & Hobbs, 1958). Groups of 8 mice received the test or a reference compound orally. Two hr after drug administration all mice were injected subcutaneously with amphetamine 15 mg/kg and then housed under crowded conditions as described by D'Arcy & Spurling (1961). After 4 hr the number of animals still alive in each group was recorded.

ACTION ON SPINAL REFLEXES

Male or female rabbits weighing 1·8–2·4 kg were anaesthetized with urethane 1·25 g/kg intravenously. In some experiments the knee jerk (monosynaptic reflex) was elicited at 10–30 sec intervals by tapping the patellar tendon (Palmer automatic knee jerk hammer). The method was similar to that described by Schweitzer & Wright (1938). In other experiments, flexor contractions of the tibialis anterior muscle (multi synaptic reflex) were elicited at 10–30 sec intervals by stimulation of the central end of the ligated ipsilateral superficial peroneal nerve with a train of rectangular pulses of 0·3–8 V strength and 0·1–1·0 msec width, at a frequency of 20–100 impulses per sec for a duration of 0·1–0·5 sec. Reflex contractions were recorded kymographically. Drugs were administered through a cannula in an external jugular vein.

ACTION ON CARDIOVASCULAR AND RESPIRATORY SYSTEMS

Effects in anaesthetized cats. Male or female cats weighing 2·8–4·2 kg were anaesthetized with chloralose 70 mg/kg intravenously after induction with 3% halothane in nitrous oxide and oxygen (3 : 1). Blood pressure was recorded from a femoral vein using a mercury manometer. Compounds were injected intravenously. Their effects on blood pressure or on the responses of the blood pressure to various vasoactive agents and occlusion

of the common carotid arteries, or both, were investigated. Respiratory rate and depth were also recorded in the anaesthetized cat using a Rubens non-rebreathing valve connected to a volume displacement recorder.

Effects in conscious renal hypertensive dogs. Male Beagle dogs weighing 12–15 kg were used which had been made hypertensive by application of rubber capsules to both kidneys 2–4 months before the experiment. Blood pressures were measured indirectly by application of a cuff to a carotid loop which had been exposed before the kidney operation. Blood pressure and heart rate were measured at half-hourly and then hourly intervals after oral administration of the test compound.

EFFECTS ON GASTROINTESTINAL TRACT

Compounds were investigated for their ability to inhibit gastrointestinal propulsion of a charcoal meal in mice (Brittain & Collier, 1958). Groups of 10 mice received the test compound orally. The charcoal meal was given 1 hr after drug administration and the mice killed 20 min later. The length of small intestine traversed by the meal was measured and expressed as a percentage of the total length of the small intestine.

DRUGS AND SOLUTIONS

4-Phenyl-1-piperidine carboxamide (AH 1932) is a white, odourless, tasteless, crystalline solid. It is sparingly soluble in water and was administered orally as a suspension in 5% gum acacia in water. Solutions for intravenous injection were prepared either in dilute acetic acid or in dimethylacetamide. Doses of drugs given in the text refer to the free base.

Results

ACUTE TOXICITY IN THE MOUSE AND RAT

The acute toxicities of AH 1932 and codeine following oral administration in the mouse and rat are respectively (LD 50 mg/kg with 95% fiducial limits mg/kg) AH 1932, 1,055 (887–1,255); codeine, 255 (199–306): AH 1932, 790 (637–980); codeine, 335 (293–382). Thus in these species codeine is about 2–3 times as toxic as AH 1932.

EFFECTS ON NORMAL BEHAVIOUR IN THE MOUSE, RAT, RABBIT, CAT AND DOG

Mouse. AH 1932, 50 mg/kg orally, had no visible effect on normal behaviour but after 100 mg/kg the righting reflex of animals was slightly impaired. Body posture, responses to a noxious stimulus, grooming and reactivity to a changed environment were all depressed by a dose of 200 mg/kg and these effects were more marked after an increase to 300 mg/kg. At the latter dose level there were also signs of stimulation such as restlessness, exophthalmous and mydriasis. Ptosis did not occur at any of the dose levels tested. The depressant effects of AH 1932 appeared to be due primarily to impaired muscular control.

Rat. No significant effects were observed following 100 mg/kg of AH 1932. After 200 mg/kg the animals were slightly restless and had raised body postures. Spontaneous locomotor activity was also increased but alertness was reduced. Similar but more pronounced effects were

seen after 400 mg/kg. At 800 mg/kg, the rats were very restless initially and had markedly elevated body postures but 25 min after drug administration severe ataxia developed rapidly and the animals became progressively more depressed with complete loss of the righting reflex and limb and body tone. However, in this state, corneal and pinnal reflexes were still present but ipsilateral spinal reflexes abolished.

Rabbit. The main effects following oral doses of 100 and 200 mg/kg AH 1932, were lowered body posture and reduced spontaneous motor activity. Slight catalepsy occurred in one animal which had received 200 mg/kg. A dose of 400 mg/kg also reduced respiratory rate. Following 800 and 1,200 mg/kg the righting reflex was almost abolished and animals were cataleptic; the corneal and pinnal reflexes were depressed but not absent. The behaviour of all animals was normal 24 hr after drug administration.

Cat. AH 1932 was more toxic in the cat than in other species investigated. Slight hind limb inco-ordination occurred after 100 mg/kg. After 200 mg/kg, ataxia developed in one animal and in the other there was marked catalepsy, spasticity and loss of the righting reflex. Doses of 400 mg/kg and 800 mg/kg abolished the righting reflex and marked spasticity and limb tremor developed. One of two animals receiving 400 mg/kg died and both animals receiving 800 mg/kg died.

Dog. No effects were observed after oral doses of 75 and 150 mg/kg. After 300 mg/kg vomiting occurred with restlessness, impairment of the righting reflex, tremor and limb incoordination. After 600 mg/kg restlessness was marked and the dogs were ataxic. Frequent hind limb collapse occurred in one animal but the righting reflex was not abolished. All animals were normal after 24 hr.

ANTITUSSIVE ACTIVITY

Conscious guinea-pig. The effects of orally administered AH 1932, codeine and morphine on coughing induced by ammonia vapour in conscious guinea-pigs are respectively (oral ED 50 mg/kg with 95% fiducial limits mg/kg): 36·8 (30·1–45·0); 42·1 (38·1–46·5); 16·9 (14·9–19·2).

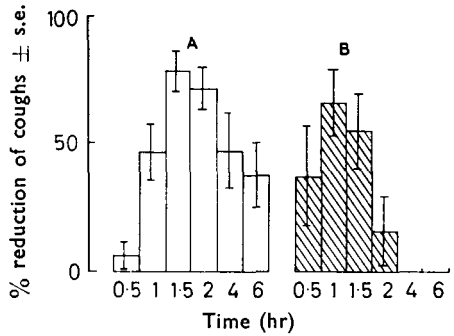

FIG. 1. The intensities and durations of the effects of AH 1932 (A) and codeine (B) on coughing induced by ammonia in conscious guinea-pigs. The ordinate gives the interval between administration of drug and exposure to ammonia vapour. The drugs were administered orally at 50 mg/kg.

Although the antitussive potency of AH 1932 was similar to that of codeine, the time courses of action of the drugs were different (Fig. 1). The onset of antitussive activity of AH 1932 was delayed but its duration of action was much longer than that of codeine. In a further experiment on conscious guinea-pigs the antitussive activity of AH 1932 and morphine were compared against coughing induced by sulphur dioxide. In this test the drugs were administered orally and AH 1932 and morphine were found to be equipotent.

Anaesthetized cat. The effects of AH 1932 and codeine in inhibiting coughing induced by mechanical stimulation of the trachea or electrical stimulation of the superior laryngeal nerve are summarized in Table 2. The record of one experiment is given in Fig. 2. In both series of experiments AH 1932 was marginally more active than codeine particularly at the lower dose levels investigated. At equipotent doses the durations of action of the drugs were similar. The effects of AH 1932 and codeine administered simultaneously to anaesthetized cats with mechanically induced coughing were additive.

TABLE 2. ACTIVITY OF AH 1932 AND CODEINE IN PREVENTING COUGHING INDUCED BY MECHANICAL STIMULATION OF THE TRACHEA OR ELECTRICAL STIMULATION OF THE SUPERIOR LARYNGEAL NERVE IN THE ANAESTHETIZED CAT

Compound	Dose mg/kg i.v.	Percentage inhibition (± s.e.) of coughing induced by	
		Mechanical stim.	Electrical stim.
AH 1932	0·125	12·5	—
	0·25	37·7 ± 3·0 (4)	36·9 ± 4·5 (4)
	0·5	64·4 ± 7·4 (4)	62·4 ± 4·7 (5)
	1·0	74·8 ± 5·7 (5)	78·9 ± 6·6 (5)
Codeine	0·25	21·3 ± 9·6 (3)	22·0
	0·5	45·3 ± 5·8 (5)	37·8 ± 9·6 (4)
	1·0	74·4 ± 5·1 (5)	77·1 ± 3·4 (5)

Figures in parentheses indicate number of determinations

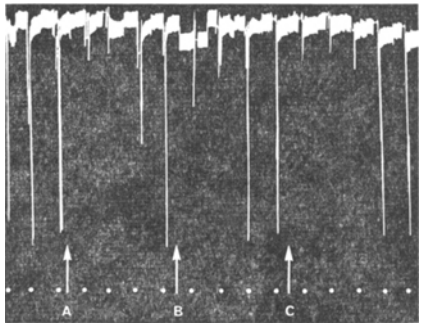

FIG. 2. Cat, 3·5 kg, pentobarbitone anaesthesia. Movements of the xiphisternum in response to stimulation of the superior laryngeal nerve (dot) at 5 min intervals. At A and B, AH 1932 injected intravenously at 1·0 and 0·5 mg/kg respectively. At C, codeine 1·0 mg/kg intravenously.

ANALGESIC ACTIVITY

The respective results of phenylquinone and tail-pinch tests in mice after oral administration of AH 1932, codeine or morphine are (ED 50 mg/kg with fiducial limits mg/kg): AH 1932 $>$ 150, $>$ 150; codeine, 12·0 (5·7–25·2); 27·6 (16·8–45·9); morphine, 3·2 (2·2–4·7); 12·8 (7·9–20·7). AH 1932 is not an effective analgesic in these tests, though codeine and morphine were highly active as expected.

ANTICONVULSANT ACTIVITY

AH 1932, 150 mg/kg orally, failed to protect animals against convulsions and deaths caused by leptazol. However, the compound possessed weak anti-convulsant activity against seizures induced by maximal electric shock and was about 1/30 as active as phenytoin.

NEUROLEPTIC ACTIVITY

AH 1932, 150 mg/kg orally, was inactive in the anti-amphetamine test. In contrast, the ED 50 for chlorpromazine in this test was 2·74 (1·55–4·85) mg/kg. It can be concluded therefore that AH 1932 has no neuroleptic activity.

ACTION ON SPINAL REFLEXES

AH 1932, 5 mg/kg intravenously, depressed contractions of a tibialis muscle elicited through a multisynaptic pathway (flexor reflex) by about 50% for 7–10 min. The depressant effect was more marked after 10 mg/kg. At these dose levels AH 1932 had little or no effect on contractions of the quadriceps femoris muscle elicited through a monosynaptic pathway (patellar reflex). These results indicate that AH 1932 possesses spinal interneuron blocking activity.

ACTION ON CARDIOVASCULAR AND RESPIRATORY SYSTEMS

Effects in the anaesthetized cat. Intravenous doses of 1–5 mg/kg AH 1932 had no significant effect on arterial blood pressure or on blood pressure changes due to vasopressor agents such as noradrenaline, tyramine nicotine and angiotensin. A higher dose, 10 mg/kg, caused variable but transient effects on the blood pressure but these effects could also be reproduced by injection of the solvent alone. This dose level also partially depressed for a short period the pressor responses to occlusion of the common carotoid arteries and to injected noradrenaline, tyramine and nicotine. AH 1932, 1–10 mg/kg, had no significant effect on heart rate and the ECG.

AH 1932 produced no marked effect on respiration. Intravenous doses of 2·5–10 mg/kg produced initial stimulation of respiratory rate and depth but again this response could usually be attributed to the solvent used. No depression of respiration was seen with any of the dose levels investigated.

Effects in conscious renal hypertensive dogs. Three hypertensive dogs received AH 1932, 50 mg/kg orally, for 3 consecutive days. The compound had no significant effect on blood pressure or heart rate nor did it cause noticeable changes in the behaviour of the animals.

EFFECTS ON GASTROINTESTINAL TRACT

The comparative effects of AH 1932 and codeine after oral administration in the mouse on gastrointestinal propulsion of a charcoal meal are illustrated in Fig. 3. In contrast to codeine, AH 1932 at high oral doses did not inhibit transport of the charcoal meal along the gastrointestinal tract.

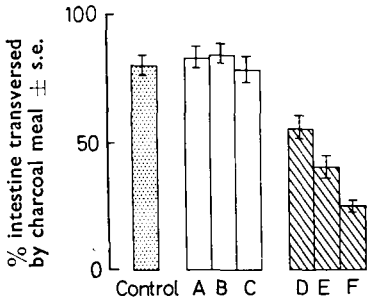

FIG. 3. The effects of AH 1932 and codeine on the gastrointestinal propulsion of a charcoal meal in conscious mice. At A, B and C, AH 1932 administered orally at 25, 50 and 100 mg/kg respectively. At D, E and F, codeine administered orally at 25, 50 and 100 mg/kg respectively. All drugs given 1 hr before, and animals killed 20 min after, the administration of the test meal.

Discussion

4-Phenyl-1-piperidinecarboxamide (AH 1932) consistently showed marked activity in preventing coughing induced in laboratory animals by chemical or mechanical irritation of the respiratory tract or by electrical stimulation of the superior laryngeal nerve. In these tests AH 1932 was as active as codeine. In guinea-pigs the action of AH 1932 was slower in onset but more prolonged than that of codeine, significant antitussive activity persisting for 6 hr after oral administration. With codeine, activity usually ceased within 2 hr. The site of action of AH 1932 is not definitely known, but it is unlikely that a peripheral inhibitory action on sensory receptors is involved since AH 1932 was equally effective in blocking coughing induced by stimulation of the central end of the superior laryngeal nerve and coughing induced by irritation of the respiratory tract. These observations suggest a central site of action.

In contrast to the morphine group of antitussive agents, AH 1932 was devoid of analgesic activity as shown by its lack of activity in the phenylquinone and tail-pinch tests in the mouse. The phenylquinone test was introduced for detecting non-narcotic analgesic activity of new compounds (Siegmund, Cadmus & Lu, 1957). However, many other types of compound, for example, narcotic analgesics, parasympathomimetics, sympathomimetics, anti-inflammatory agents and monoamineoxidase inhibitors, inhibit writhing induced by phenylquinone (Hendershot & Forsaith, 1959; Brittain & others, 1963). Since AH 1932 was inactive in the phenylquinone test it may be inferred that the drug is probably also devoid of the many types of activity outlined above. The lack of analgesic activity in

AH 1932 coupled with its obvious chemical dissimilarity to morphine would also indicate that problems of tolerance and addictive liability are unlikely to occur with this compound.

Behavioural studies with AH 1932 indicated a curious mixture of stimulant and depressant effects, the latter predominating at higher doses. It was thought that the depressant effects were due mostly to impaired muscular control. The drug depresses the multisynaptic flexor reflex but not the monosynaptic patellar reflex, showing this impairment to be caused by spinal interneuron blockade. These results also rule out action of AH 1932 on the myoneural junction, on skeletal muscle and on conduction in peripheral nerves including the lower motoneurons involved in coughing. The doses necessary to cause interneuron blockade greatly exceed those which cause antitussive activity and it is therefore unlikely that the interneuron blocking effects of AH 1932 would limit its use as an antitussive agent.

AH 1932 had no effect on respiration; an advantage over morphine and related compounds, which are known to depress respiration (Kreuger Eddy & Sumwalt, 1941; Wikler, 1950; Loeschcke, Sweel & others, 1953). A further advantage of AH 1932 over codeine might be its lack of action on the gastrointestinal tract, codeine being well known to cause constipation (Meyler, 1966).

The cardiovascular actions of AH 1932 are slight. At high dose levels in the anaesthetized cat there was a weak α-adrenergic blocking action. However, in conscious hypertensive dogs even higher doses given orally had no noticeable effects on the cardiovascular system. Cardiovascular effects are unlikely to limit the use of the drug as a cough suppressant.

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