A COMPARATIVE STUDY OF THE SPASMOLYTIC ACTIVITIES OF OCTAVERINE, PERPARINE AND PAPAVERINE

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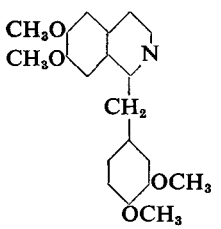
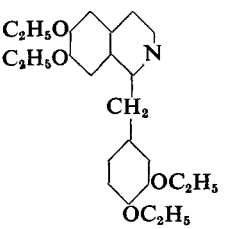
PAPAVERINE has been widely used for peripheral thrombosis and embolism, myocardial infarction, angina pectoris, bronchial asthma, hypertensive states, renal and biliary colic and other conditions in which relaxation of smooth muscle is desired. The alkaloid occurs in opium in association with morphine and this accounts for the noticeable predisposition to bias against its prolonged use as a therapeutic agent in spite of the fact that no case of addiction to papaverine has been recorded. Since the original synthesis of Pictet and Gams[1] a number of synthetic routes have been evolved[2] and at the present time papaverine is almost exclusively manufactured from vanillin or veratrol.

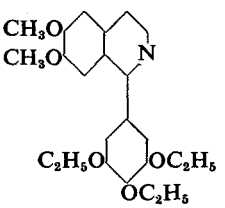
In the search for compounds with lower toxicity and greater, or more selective, activity many analogues of papaverine have been prepared. Apart from those synthetics containing a substituent in the 3-position of the quinoline nucleus, these fall into two categories (i) compounds containing the 1-benzyl*iso*quinoline nucleus of papaverine but in which the peripheral alkoxy groups are varied and (ii) compounds in which the 1-dialkoxybenzyl group in papaverine is replaced by a 1-polyalkoxyphenyl group. Representatives of these two classes are perparine and octaverine, obtained respectively by cyclodehydration and subsequent dehydrogenation of *N*-(3:4-diethoxyphenylacetyl)-3:4-diethoxyphenylethylamine and *N*-(3:4:5-triethoxybenzoyl) homoveratrylamine.



Papaverine



Perparine



Octaverine

The literature on perparine is extensive[3] although no direct comparison of its pharmacodynamic properties with those of papaverine exists. Octaverine, on the other hand, has received scant mention; Ellinger, Koschara and Seeger[4] described the compound as a synthetic with comparatively low toxicity having a reliable and persistent action upon smooth musculature.

The purpose of the present work was to compare the spasmolytic properties of octaverine and perparine with those of papaverine under identical conditions. The following properties have been examined (i)

mouse acute toxicity, (ii) action on spontaneous contractions of isolated rat uterus, (iii) inhibition of spasm in isolated guinea-pig ileum induced by acetylcholine bromide, histamine, barium chloride and pilocarpine, (iv) and bronchodilator action as shown by protection against histamine induced bronchospasm in guinea-pigs. The activities of the 3 alkaloids on the circulatory and respiratory systems, the activities in counteracting adrenaline induced hypertension and their effect upon the perfused isolated heart will be reported in a further communication.

The solutions of the alkaloids used were 0·2 per cent. octaverine isethionate, 5·0 per cent. perparine isethionate and 1 per cent. papaverine hydrochloride. For the higher toxicity range a 2·0 per cent. solution of octaverine methanesulphonate was employed in order to avoid injecting excessive volumes of water; this solution remained clear provided it was kept at 37° C. All doses of these solutions are expressed as the corresponding amounts of the free alkaloid base. In the spasmolytic work on uterus and ileum, in order to eliminate any possibility of alteration in tone being due to the *p*H effect[5], it was ascertained that the addition of the maximum concentrations of the alkaloid solutions to the Ringer-Locke and Tyrode solutions caused no change in the *p*H values.

Mouse Acute Toxicity

The alkaloids were administered to 18–22 g. mice of mixed sex by the intraperitoneal route in closely spaced doses. Groups of 4 mice were used from each dose, approximately 40 mice being used for the determination of each toxicity range; the animals were observed for 7 days after the single dose. The results are shown in Table I.

TABLE I

Number of mice	Dose mg./kg.	Reflexes	Alertness	Convulsions	Survival per cent.
		Octaverine			
4	50	Normal	Normal	−	100
4	100	"	"	−	100
4	160	Diminished	Drowsy	−	100
4	180	"	"	−	100
4	190	Lateral decubitus	Sleeping	−	100
4	200	" "	"	−	50
4	210	" "	"	+	50
4	240	" "	"	+	0
		Perparine			
4	50	Normal	Quiet	−	100
4	100	"	Drowsy	−	100
4	160	Lateral decubitus	Sleeping	−	100
4	170	" "	"	−	100
4	180	" "	"	+	100
4	190	" "	"	+	100
4	200	" "	"	+	75
4	210	" "	"	+	50
4	220	" "	"	+	50
		Papaverine			
4	30	Normal	Drowsy	−	100
4	60	Diminished	"	−	100
4	80	Strongly diminished*	Sleeping	+	100
4	100	Lateral decubitus	"	+	100
4	110	" "	"	+	100
4	120	" "	"	+	50
4	130	" "	"	+	50
4	140	" "	"	+	0

* 2 Mice had lateral decubitus.

SPASMOLYTIC ACTIVITIES

Compound	Approximate LD50	Minimum convulsive dose
Octaverine ..	200 mg./kg.	100 per cent. of LD50
Perparine ..	210 mg./kg.	85 per cent. of LD50
Papaverine ..	120 mg./kg.	65 per cent. of LD50

The acute toxicities of these alkaloids have not previously been compared in any one animal and the figures recorded in the literature are conflicting. For example, Issekutz, Leinzinger and Dirnier[6] record that the fatal dose of papaverine for the cat is 120 mg./kg. by subcutaneous injection and for the mouse 150 mg./kg. (route of administration not recorded). The same authors state that the minimum lethal dose of perparine for rats was 500 mg./kg. (intraperitoneal) and 1500 mg./kg. (peroral); Langecker and Starkenstein[7] state that in mice 200 mg./kg. of perparine (intraperitoneal) caused convulsions and that 550 mg./kg. caused death. Ellinger *et al.*[4] record that lateral decubitus ensues in mice after an intraperitoneal dose of 250 mg./kg. of octaverine but that the animal may still be awakened; that loss of contact sensation does not occur until the dose is increased to 300 mg./kg.; and that the median lethal dose is in the neighbourhood of 380 mg./kg. The present work indicates that the toxicities of octaverine and perparine are approximately one-half that of papaverine as judged by the combined LD50 figures, the minimum convulsive doses and the minimum doses required to cause diminution of reflexes and lateral decubitus.

ACTION OF THE ALKALOIDS ON SPONTANEOUS CONTRACTIONS OF ISOLATED RAT UTERUS

The isolated uterus was immersed in oxygenated Ringer-Locke solution (50 ml.) at 37° C. and the amplitude of the movement registered on the kymograph using a lever giving a 3-fold magnification. The spasmolytic activity of the alkaloids was assessed by determining the minimum concentration in the perfusing solution required to produce 100 per cent. inhibition of the spontaneous contraction. Each concentration was allowed to act for 2 minutes and then the tissue was given a sufficient number of standard washes for the spontaneous contractions to return to normal; a standard wash consisted in emptying the bath, refilling with Ringer-Locke solution and allowing this to stand for 2 minutes before again emptying. The average results from 15 rat uteri are shown in Table II from which it is evident that octaverine is 4 times, and perparine twice, as active as papaverine. Figure 1 comprises three typical kymograph records.

TABLE II

Compound	Minimum concentration to effect 100 per cent. inhibition of contractions
Octaverine	4 mg./l.
Perparine	8 mg./l.
Papaverine	16 mg./l.

It was repeatedly observed that uterus which had been treated with the above doses of octaverine and papaverine required 3 standard washes in order to bring back the rhythmic contractions to normal; with perparine (8 mg./l.), however, 9 standard washes were necessary. This may be due to enhanced adsorption upon muscle tissue in the case of perparine. It is of interest that this persistence of the decrease in tone effected by perparine was remarked upon by Langecker and Starkenstein.[7] In addition, it is noteworthy that perparine, as well as inhibiting the spontaneous contraction, produces profound relaxation at the high dose; no such effect was observed with either octaverine or papaverine.

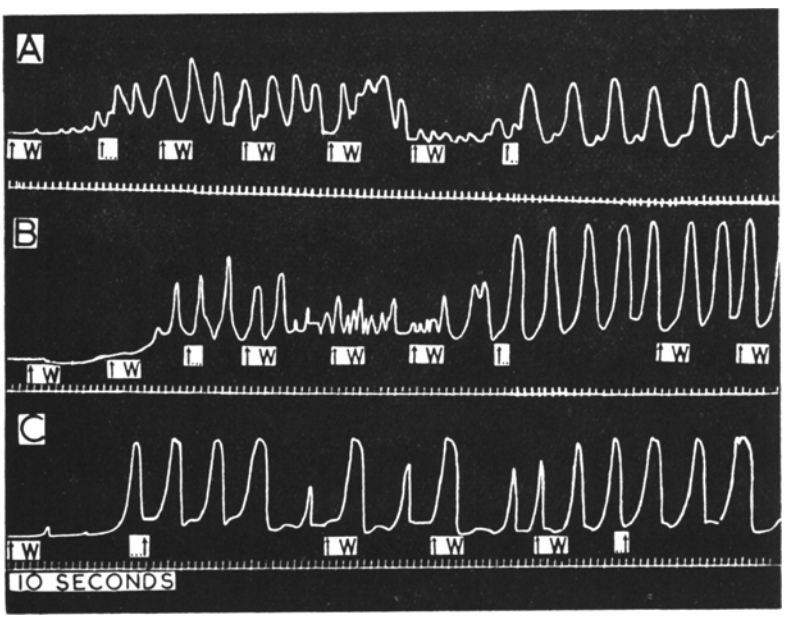


FIG. 1. Action of octaverine, perparine and papaverine on spontaneous contractions of isolated rat uterus. The record reads from right to left.

A. Octaverine ↑.. = 2 mg./l. ↑... = 4 mg./l.
B. Perparine ↑.. = 4 mg./l. ↑... = 8 mg./l.
C. Papaverine ↑.. = 8 mg./l. ↑... = 16 mg./l.
W = Wash

INHIBITION OF INDUCED SPASM IN ISOLATED GUINEA-PIG ILEUM

A 3-cm. strip of freshly isolated guinea-pig ileum which showed no spontaneous activity was perfused with a measured volume of oxygenated Tyrode solution at 37° C. and a standard reproducible spasm of at least 2 minutes' duration provoked by injecting a selected amount of the spasm producing substance into the bath. Measured amounts of the alkaloids were then added to the perfusing liquid in order to determine the minimum concentration of alkaloid required to abolish the spasm. Spasmogens used were acetylcholine bromide, histamine, barium chloride

and pilocarpine. The results obtained from work with 36 strips of ileum (Table III) show that the 3 alkaloids have the same activity against barium-induced spasm. Against histamine-induced spasm, octaverine and papverine have the same activity; perparine possesses one-half of this activity. Octaverine and papaverine have the same activity against spasm provoked by pilocarpine; perparine possesses double this activity. Since the

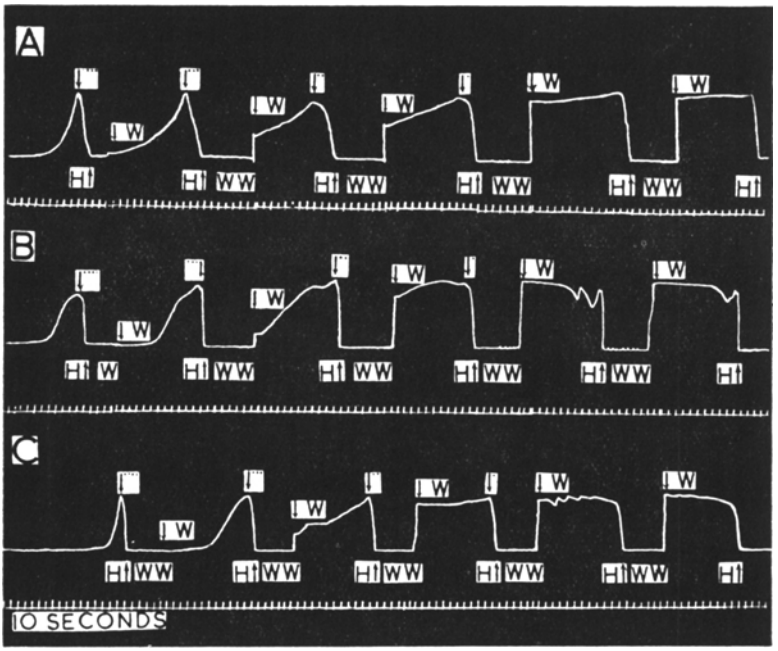


FIG. 2. Action of octaverine, perparine and papaverine on histamine induced spasm in isolated guinea-pig ileum. The record reads from right to left.

A. Octaverine ↓. = 1·25 mg./l. ↓.. = 2·5 mg./l. ↓... = 5 mg./l. ↓.... = 10 mg./l.

B. Perparine ↓. = 2·5 mg./l. ↓.. = 5 mg./l. ↓... = 10 mg./l. ↓.... = 20 mg./l.

C. Papaverine ↓. = 1·25 mg./l. ↓.. = 2·5 mg./l. ↓... = 5 mg./l. ↓.... = 10 mg./l.

H = histamine 0·2 mg./l. W = wash

spasm induced by acetylcholine bromide (0·027 mg./l.) was abolished by the addition of atropine in a concentration of 0·016 mg./l. it is evident that none of the 3 alkaloids has appreciable ability to block the muscarinic action of acetylcholine. Figure 2 shows a typical set of 3 kymograph records against histamine spasm.

After each dose of spasmolytic alkaloid the tissue was given a sufficient number of standard washes (*v.s.*) to restore it to the normal state as shown by the elicitation of the same response by a further dose of the same spasmogen. It was observed after spasm due to acetylcholine

TABLE III

Spasmolytic agent	Spasmogen			
	Acetylcholine bromide 0·027 mg./l.	Histamine 0·2 mg./l.	Barium chloride 200 mg./l.	Pilocarpine 6·6 mg./l.
Octaverine ..	52 mg./l.	5 mg./l.	10 mg./l.	26 mg./l.
Perparine ..	26 mg./l.	10 mg./l.	10 mg./l.	13 mg./l.
Papaverine ..	26 mg./l.	5 mg./l.	10 mg./l.	26 mg./l.

bromide, barium chloride and pilocarpine and dissolution of the spasm by all 3 alkaloids that 3 washes were required to restore normal tone. After spasm provoked by histamine, and lysis of spasm by octaverine and papaverine, 3 standard washes were required; after relief of histamine spasm by perparine, however, 24 standard washes were required before the tissue would give a normal response to histamine.

Bronchodilator Activity *in vivo*: Protection Against Histamine Induced Bronchospasm in Guinea-pigs

A modification of the method of Loew[8] was used. The guinea-pigs were placed 2 at a time in a 6-l. glass desiccator, the lid closed and 2·5 mg. of histamine dihydrochloride (0·25 ml. of 1 per cent. aqueous solution) sprayed in as a mist through the side inlet from a fine glass atomiser. The time interval between administration of histamine and development of severe bronchospasm was noted; if no spasm ensued the animals were removed after 10 minutes. 10 animals were used for each dose of each alkaloid and the activities of the latter expressed as the percentage of the animals which did not develop bronchospasm.

Orally administered doses of the 3 alkaloids (25 mg./kg.) failed to protect against the bronchospasm 30 minutes, 60 minutes and 90 minutes after the dose of alkaloid. Papaverine (50 mg./kg.) also failed to protect under these conditions; both octaverine and perparine (50 mg./kg.) gave protection to 25 to 50 per cent. of the animals although the "protected" animals had intermittent spasm of the neck muscles throughout the 10 minutes observation period and showed a certain amount of respiratory distress.

Summary

1. A comparative study has been made of the toxicities and spasmolytic activities of papaverine and 2 synthetic analogues, octaverine and perparine.

2. Octaverine and perparine have about one-half of the acute toxicity of papaverine, as shown by the combined LD50 figures, the minimum convulsive doses and the minimum doses required to diminish reflexes and establish lateral decubitus in mice.

3. Octaverine is 4 times, and perparine twice, as active as papaverine in suppressing the spontaneous contractions of rat uterus.

4. All 3 alkaloids have the same activity against barium chloride-induced spasm in isolated guinea-pig ileum. Octaverine and papaverine possess the same activity against spasm provoked by histamine or by

pilocarpine; perparine has one-half of the activity of papaverine against histamine spasm and double the activity of papaverine against pilocarpine spasm.

5. Orally administerd papaverine has no action against histamine-induced bronchospasm in guinea-pigs; both octaverine and perparine possess a definite although small activity under comparable conditions.

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