Evaluation of Antispasmodic Activity in the Intact Dog

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Results of a method developed to determine the comparative effectiveness of the active components of an antispasmodic formula and their combination in the surgically intact animal are presented. These confirm established relative potencies of the compounds involved and indicate that the combination of the formula under investigation represents a potentiating union.

THE QUALITATIVE and quantitative effects of atropine and chemically related cholinergic blocking agents have been demonstrated by numerous investigators using both *in vivo* and *in vitro* pharmacologic procedures. In most instances, some sort of surgical intervention of the gastrointestinal tract has been involved in the *in vivo* studies to date. Excellent *in vitro* biological assays such as those reported by Luduena and Lands are available (1). Using ileal strips, these investigators have achieved procedures of general acceptance in the pharmacologic screening of cholinergic blocking agents.

One of the problems confronting *in vivo* methods has been that involved with the tachyphylactic response to the more potent cholinergic blocking agents, evidenced in the progressive depression of gastrointestinal sensitivity and described by Seevers, *et al.* (2), in 1954 and by Quigley, *et al.* (3), in 1937. Since the reliability of a biological assay is known to be dependent upon the stability of the dose-response relationship, the tachyphylactic response here encountered constitutes a barrier to the successful development of a biological assay method for the aforementioned agents.

The quantitative determination of the cholinolytic activity of atropine and its belladonna relatives individually and in combination *in vivo* constitutes the basis of this investigation.

EXPERIMENTAL

Pressure changes occurring in the sensing element, a rubber sheath, in response to gastrointestinal movements were transmitted by means of its partially encased Cantor tube (D-111, 12, French) to a sensitive Sanborn transducer where these changes were converted to electrical impulses. The electrical impulses were then, after amplification, conveyed to a Sanborn two-channel recorder.

Along with measurement of gastrointestinal tract activity of the test animal, the respiratory activity was recorded concomitantly by means of a pneumograph, a second Sanborn transducer and Sanborn strain gauge amplifier being employed for this recording.

The test animals were mongrel dogs of either sex ranging in weight from 11.85 to 18.0 Kg. During the study they were fed once daily and supplied with water *ad libitum*. Each dog was anesthetized before each test with 30 mg./Kg. of pentobarbital sodium administered intravenously. The level of anesthesia that was achieved was just insufficient to abolish the swallowing reflex. No dog was used more often than every 48 hours for the testing of these compounds.

In all the tests, the Cantor tube reached approximately the same level in the gastrointestinal tract. At the completion of this series of tests a dog was sacrificed to reveal that the rubber sheath was located in the pyloric portion of the stomach.

All drugs were administered intravenously by means of an in-dwelling 18-gauge hypodermic needle, which was attached by a rubber tube to a 50-ml. buret containing 0.9% saline solution.

In the series of tests involving the response of the gastrointestinal tract to methacholine chloride, tachyphylaxis was not observed.

In view of the possibility of achieving a complete block with atropine sulfate and the chemically related cholinergic blocking agents of belladonna, the range values of the blocking dose of atropine sulfate were determined experimentally prior to the study to be 0.067 to 0.10 mg./Kg. Ten minutes after the intravenous administration of 0.10 mg./Kg. of atropine sulfate, no increase in gastrointestinal activity was achieved with an indefinitely large dose of methacholine chloride, although profuse salivation was evidenced.

In preliminary experiments, it was noted that the greater the dose of the spasmogen, methacholine chloride, the greater the response and the greater the duration of the response. Termination of the response was assumed when the base line of the recording returned approximately to its predose level. The mean average duration of the response to methacholine chloride, according to the above criterion, was 8 minutes, with a range of 4–10 minutes. To allow a margin of safety, the spasmogen was administered at 15-minute intervals throughout the entire series of the tests. Thus, cumulative effects of methacholine chloride were avoided.

The onset of atropine and hyoscyamine activity, which was indicated by a fall in the base line of the graph, was shown to occur within 5 minutes of the

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intravenous administration of these cholinergic blocking agents. The establishment of the atropine or the hyoscyamine partial cholinergic block of the gastrointestinal tract was demonstrated in every instance within 10 minutes following the intravenous administration of these agents. The onset of hyoscine activity was more variable than that of atropine or hyoscyamine, and the establishment of the partial cholinergic block of hyoscine required at least 30 minutes following the intravenous administration of the drug.

From the preliminary studies, it became evident that the antispasmodic activity of atropine and related belladonna alkaloids is sufficiently enduring that cumulative effects would almost certainly occur if several doses of antispasmodic were administered during the course of one determination. This would, of course, make difficult or impossible the evaluation of the effectiveness of a single dose of antispasmodic. Consequently, the individual alkaloids and their combinations were given in this method only one dose per determination.

Methacholine chloride, the spasmogen, was administered in varying doses with the intent of obtaining a methacholine chloride$_{50}$, or Me$_{50}$, a dose of methacholine chloride the response to which is reduced by the antispasmodic to 50% of that to a preantispasmodic dose of this drug. The extent of the inhibition of methacholine chloride was determined and expressed as per cent of the preantispasmodic response.

A minimum of three doses was employed in order to obtain a Me$_{50}$: a high dose with an inhibition of less than 50%, a low dose with an inhibition of more than 50%, and a middle dose with an inhibition of approximately 50%. Each postantispasmodic dose of methacholine chloride in mcg./Kg. was plotted against the corresponding per cent reduction of the preantispasmodic response to methacholine chloride on logarithmic probability paper. The line to fit these data was drawn by inspection and the Chi^2 test was applied to determine the goodness of fit. From this line of the plotted data the Me$_{50}$ was read directly.

Throughout a particular determination, only one preantispasmodic dose of methacholine chloride was used, and the per cent reduction of the postantispasmodic methacholine responses was determined from this value.

The Me$_{50}$ of atropine sulfate at 0.030 mg./Kg. was calculated to be 9.25 mcg./Kg. To assess a relative activity with respect to the other antispasmodics and a combination of belladonna alkaloids[1] (CBA) the Me$_{50}$ 9.25 of each of these agents was determined. A minimum of three doses of antispasmodic was employed in order to obtain an antispasmodic dose with an Me$_{50}$ 9.25: a dose with an Me$_{50}$ above 9.25, a dose with an Me$_{50}$ below 9.25, and a dose with an Me$_{50}$ between these values. The log of each Me$_{50}$ was plotted against the log of the corresponding dose of the antispasmodic. From the resulting linear relationship, the Me$_{50}$ 9.25 of that particular antispasmodic or combination of antispasmodics was read directly from the graph and was calculated from the regression equation.

Each antispasmodic was assigned a relative potency value which was based on the reciprocal of its Me$_{50}$ 9.25 value multiplied by the Me$_{50}$ 9.25 of atropine sulfate, 0.030 mg./Kg. Thus atropine was given a value of unity and the other agents, including CBA, were listed according to their relative potencies as multiples of this number.

The individual Me$_{50}$ 9.25 doses of the three active components were weighted according to the relative concentrations of each in the combination, CBA. The sum of these weighted means was then divided by the sum of the relative concentrations of the components and the quotient obtained was considered to represent the calculated Me$_{50}$ 9.25 of CBA. The actual or the experimental Me$_{50}$ 9.25 value of CBA was obtained as were the component values. Comparison of the calculated and the experimental values for CBA effectiveness indicates that the combination constitutes an activity greater than the summed activity of the components.

DISCUSSION

This investigation has produced a method for evaluating the antispasmodic effectiveness of anti-

TABLE I.—THE ANTISPASMODIC ACTIVITY OF BELLADONNA ALKALOIDS

Alkaloid	Dose, mg./Kg.	Me$_{50}$, mcg./Kg.	A[a]	B[b]
Atropine sulfate	0.030	9.25	4.22	12.8
			8.44	55.3
			16.88	70.2
			25.32	88.94
Hyoscyamine sulfate	0.010	4.7	3.13	14.7
			6.25	68.8
			9.38	90.0
Hyoscyamine sulfate	0.0125	6.8	3.67	7.5
			5.51	22.5
			7.34	65.0
Hyoscyamine sulfate	0.0150	14.25	4.41	15.6
			8.82	42.6
			17.6	53.0
			26.46	64.8
Hyoscine hydrobromide	0.005	7.9	2.5	10.0
			5.0	20.2
			10.0	59.4
Hyoscine hydrobromide	0.005	9.2	3.75	6.0
			6.25	44.0
			12.5	78.0
Hyoscine hydrobromide	0.007	6.2	3.68	36.7
			5.51	43.3
			11.0	73.3
Hyoscine hydrobromide	0.007	6.2	3.13	15.0
			6.25	57.5
			12.5	86.4
Hyoscine hydrobromide	0.0073	5.6	2.8	7.8
			5.6	44.6
			8.2	79.2
Hyoscine hydrobromide	0.009	7.9	4.72	2.9
			9.45	17.0
			15.7	33.3
			23.9	57.0
CBA	0.009	4.5	3.67	35.5
			5.5	61.9
			7.35	85.75
CBA	0.012	14.2	7.35	25.5
			14.7	50.0
			18.5	61.4
			22.1	68.2
CBA	0.015	19.5	5.0	10.0
			10.0	19.5
			16.65	59.7

[1] Kindly supplied as Donna by A. H. Robins and Co., Inc., Richmond, Va.

[a] A = Methacholine chloride mcg./Kg. [b] B = Per cent reduction.

TABLE II.—COMPUTATION OF CALCULATED VALUE OF THE Me_{50} 9.25 MCG./KG. FOR DONNA (CBA)

Alkaloid	Concentration in Donna	Actual Me_{50} 9.25 mcg./Kg.
Hyoscyamine sulfate	1037 × 0.01316	= 13.6469
Atropine sulfate	194 × 0.03000	= 5.8200
Hyoscine hydrobromide	65 × 0.00685	= 0.4453
	1296	19.9122[a]

[a] $\frac{19.9122}{1296}$ = 0.01536 mg./Kg. of Donna is the calculated value of the Me_{50} 9.25 mcg./Kg.

TABLE III.—ANTISPASMODIC ACTIVITY IN COMPARISON WITH ATROPINE SULFATE

Drug	Me_{50} 9.25 mcg./Kg.	Potency Compared to Atropine Sulfate
Atropine sulfate	0.0300	1.0
Hyoscyamine sulfate	0.01316	2.28
Hyoscine hydrobromide	0.00685	4.38
CBA	0.01120	2.68

cholinergic agents generally. The potency of such agents may be assessed in the procedure on the basis of comparison with an established standard. The advantage claimed for the method is that it is carried out in the intact dog without the necessity for surgical intervention in the placement of the sensing element. Thus, the same group of animals can be used repeatedly and without lengthy preparation in the determination involved.

The method developed has made possible a comparison of the relative effectiveness of the individual components of the combination CBA with the relative effectiveness of the combination. The results obtained have indicated potentiation of action in the combination.

Although atropine, hyoscyamine, and hyoscine were administered intravenously, it is very likely that similar results would be produced by oral administration, since these alkaloids are well absorbed from the gastrointestinal tract. Synthetic blockers should be studied, to be sure, by several routes of administration, since an agent which shows considerable cholinergic blocking activity parenterally could fail to achieve clinical usefulness because of poor absorption from the gastrointestinal tract.

Another possibility for further application of the method involves the direction of the alteration of the concentrations of the various alkaloids in such combinations as CBA in order to achieve an optimum combination of the component alkaloids, *i.e.*, one providing the greatest potentiation of the therapeutic benefits of the components consistent with the lowest incidence and least severity of untoward side effects.

SUMMARY

A biological assay employing the surgically intact dog is used to determine the relative activity of atropine sulfate, hyoscyamine sulfate, and hyoscine hydrobromide both alone and in combination on the gastrointestinal tract.

Indication of potentiation in the antispasmodic activity of CBA is provided by a comparison of the experimentally determined activity with the activity calculated from the activities of the components.

REFERENCES

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(3) Quigley, J. P., *Proc. Soc. Expll. Biol. Med.*, **36**, 450 (1937).

ERRATUM

In the paper titled "Effects of Ionizing Radiation on Two Gelatin Fractions I. Material Preparation, Dosimetry, and Acid-Base Behavior" (1), the caption for Fig. 3 should be revised to read: ". . . after 5% dispersions of each were subjected to an irradiation dose of 2.0 Mrads."

(1) Prusak, L. P., and Sciarrone, B. J., THIS JOURNAL, **51**, 1046(1962).