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Evaluation of Antispasmodic and Related Activity in the Intact Dog

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Abstract □ This is a report of the development of a method designed to assess concomitantly in the intact dog the antispasmodic activity of a compound, its effect on salivation, pupil size, heart rate, urinary activity, gastric acidity, gastric volume, blood pressure, and respiration. In the method the Me_{50} value of the antispasmodic is obtained graphically by plotting the response to each of three (or more) different postantispasmodic doses of methacholine chloride in terms of percent of the response to preantispasmodic dose(s) of methacholine. Such an Me_{50} value was obtained for each of three different parameters: motility of the digestive tract, motility of the urinary tract, and salivary secretory activity. The effects on other parameters which lend themselves to less exacting measurements are evaluated with considerably less precision. By supplying a means for comparing the effects of an antispasmodic compound on each of these three parameters under identical circumstances, the method makes possible the determination of the relative side-effect liability of that compound with respect to dryness of the mouth and urinary retention. Since the relative side-effect liability, expressed as a ratio, differs from one side effect to another and from compound to compound, the method provides an indication of the acceptability or usefulness of the compound.

Keyphrases □ Anticholinergic agents—bioassay method □ Bioassay method, anticholinergics—surgically intact dog □ Me_{50} value determination—anticholinergics □ Methacholine spasmodic activity—anticholinergic agent effect

The employment of anticholinergic agents in the therapy of the hypersecretory and hypermotile tract has dictated the adoption of various methods for the bioassay of such compounds. The majority of these represent *in vivo* methods such as those described by Ingelfinger (1) in 1943 and Code *et al.* (2) in 1952. For the most part these are involved with some sort of surgical manipulation of the animal, which may of itself alter the results obtained. In spite of the fact that the incidence of peptic ulcer is chiefly attributable to excessive secretion and/or motility, these methods have been concerned for the most part with the ability of the compounds undergoing potency testing to inhibit or abolish motor activity of the tract.

Since this is but one of the etiologic factors in the condition itself or the predisposition to it, and since the anticholinergic-type agent, being autonomic, exerts a variety of effects, the overall action of the agent in question becomes of considerable significance insofar as therapeutic usefulness is concerned. The toxicity of any drug is of great importance, but beyond this it is necessary to view the effectiveness of the drug against its side-effect liability. It goes without saying that the most effective drug is not necessarily the most useful. Accordingly a multiparameter assay of these anticholinergic agents which will assess not only antimotility and antisecretory activity but also side-effect propensity in the surgically intact dog is indicated if the whole action of these compounds in the body is to be projected accurately. Such an assay is necessarily adapted to the evaluation of neurotropic antispasmodic agents and in its development four agents in this category, available on the market, were selected for comparison with the neurotropic standard, atropine.

Turkanis (3) in 1963 described an assay method which may be used to compare the potencies of antispasmodic compounds in the surgically intact dog. The method was based on the establishment of a unit of antispasmodic activity: the Me_{50}, a computed quantity of methacholine, the response to which is reduced by the antispasmodic to 50% of the original value. This method, expanded and improved by Ryan (4) in 1964 and Benoit (5) in 1965, provided the basic antispasmodic procedure for this study. Since the work of Ryan had shown that the use of pentobarbital as an anesthetic might have an adverse effect on the results obtained, a combination of chloralose and sodium thiopental was employed in subsequent investigation.

EXPERIMENTAL

In the method presented, motility changes in the digestive tract are picked up by an inflated rubber sheath at the end of a polyethylene tube and transferred by the tube to a transducer and amplifier through which they are recorded. The rubber sheath is inserted

Table I—Ratios Reflecting Relationship Between Spasmolytic Activity and Side-effect Liability (Typical Determination)

Atropine Sulfate, 30.00 mcg./kg.[a]	
Dog I	
Gastrointestinal activity Me_{50}	7.40 mcg./kg.
Urinary activity Me_{50}	12.80 mcg./kg.
Salivary activity Me_{50}	25.00 mcg./kg.
Urinary activity Me_{50}/GI activity Me_{50} = 12.80/7.40 = 1.73	
Salivary activity Me_{50}/GI activity Me_{50} = 25.00/7.40 = 3.38	

[a] The dose of atropine sulfate (administered as a single dose) was selected on the basis of its inhibition of the response to each of a series of methacholine doses. With this quantity of atropine, it was possible to select doses of methacholine the responses to which made possible the computation of the Me_{50} equivalent (for each parameter). The first of the postantispasmodic doses of methacholine was administered in each instance 30 min. after the atropine.

through the oral cavity immediately prior to the determination and is then inflated with air to a pressure which is the same for each determination.

Salivary secretion is recorded by means of an open, fluid-filled system having at one end a (hypodermic) needle-tipped polyethylene tube which is attached to a 91.4-cm. (3-ft.) length of glass tubing, the other end: the latter affixed to a meter stick whose gradations indicate flow increase. The needle-tipped portion of the polyethylene tubing is inserted in Wharton's duct.

Urinary bladder activity is assessed by means of a fluid, air-filled system extending from the tip of the urethra-inserted polyethylene tubing just inside the bladder to the pressure transducer in contact with an amplifier-recorder.

Table II—Ratios of Means of Me_{50}'s Obtained in Different Determinations in the Same Animal[a] After Glycopyrrolate 8.00 mcg./kg.[b]

GI Me_{50}, mcg./kg.	Urinary Me_{50}, mcg./kg.	Salivary Me_{50}, mcg./kg.
Dog I		
7.50	8.55	14.80
7.40	8.00	10.40
6.40	7.30	7.20
5.90	7.00	6.40
4.11	5.60	
4.10		
4.10		
$\bar{X}$ 5.38	7.29	9.70
($SE \pm 3.21$)	($SE \pm 2.24$)	($SE \pm 7.63$)
Urinary Mean Me_{50}/GI Mean Me_{50} = 7.29/5.38 = 1.35		
Salivary Mean Me_{50}/GI Mean Me_{50} = 9.70/5.38 = 1.80		
Dog II		
6.40	16.50	16.20
6.10	13.50	11.20
6.10	13.50	11.20
4.70	11.20	10.40
4.15	9.00	
	8.60	
	7.40	
	5.90	
	5.45	
	4.35	
$\bar{X}$ 5.34	9.10	12.60
($SE \pm 2.16$)	($SE \pm 4.49$)	($SE \pm 6.28$)
Urinary Mean Me_{50}/GI Mean Me_{50} = 9.10/5.34 = 1.70		
Salivary Mean Me_{50}/GI Mean Me_{50} = 12.60/5.34 = 2.35		

[a] Me_{50} values listed were obtained at different times in the same animal, an interval of two to several days being represented. The two dogs selected are representative of approximately 20 animinals. [b] The dose of glycopyrrolate (administered as a single dose) was selected on the basis of inhibition of the response to each of a series of methacholine doses. With this quantity of glycopyrrolate, it was possible to select doses of metholine the responses to which made possible the computation of the Me_{50} equivalent (for each parameter). The first of the postantismodic doses of methacholine was administered 30 min. after the dose of glycopyrrolate.

Table III—Ratios of Means of Me_{50}'s Obtained in Different Determinations in Different Animals After Poldine Methylsulfate, 12.00 mcg./kg.

GI Me_{50}, mcg./kg.	Urinary Me_{50}, mcg./kg.	Salivary Me_{50}, mcg./kg.
Dog I		
7.65	7.00	16.40
4.65	6.10	13.80
4.55	4.10	8.20
4.10		
3.60		
Dog II		
	10.00	13.50
	7.30	
Dog III		
6.70	11.40	13.00
	7.30	12.70
	6.10	
Dog IV		
		11.40
		14.50
$\bar{X}$ 5.21	7.41	12.94
($SE \pm 3.18$)	($SE \pm 4.60$)	($SE \pm 9.60$)
Urinary Mean Me_{50}/GI Mean Me_{50} = 7.41/5.21 = 1.42		
Salivary Mean Me_{50}/GI Mean Me_{50} = 12.94/5.21 = 2.48		

Blood pressure is determined by means of a femoral artery puncture. The pressure of the blood at the tip of the inserted needle comes in contact with the slow-flowing heparin-saline solution of the attached polyethylene tubing and is brought to bear on a transducer connected to an amplifier-recorder. Heart rate is read from the blood pressure record.

Respiratory changes are picked up by a pneumograph and are recorded after being brought through a transducer and amplifier.

Pupil changes are estimated through a short piece of flanged glass tubing which is used to bring the cornea into view. An etched magnifying lens is employed in the estimation.

All measurements are made with as little interference with the normal function of the animal as possible, each time the same way.

For the evaluation of gastric secretory activity, gastric samples are withdrawn at specified intervals following drug administration and titrated against a standard sodium hydroxide solution. The withdrawals are made through polyethylene tubing inserted by way of the oral cavity just prior to the beginning of the series of withdrawals and kept in position throughout the series. The gastric volume withdrawn by syringe is measured to the nearest tenth of a milliliter and recorded thus.

The method is based upon the responses to various doses of methacholine chloride before and after the antispasmodic and the differences in the magnitude of the inhibition of the response to different individual doses of spasmogen by the same dose of antispasmodic (3). Previous work by Ryan (4) and Benoit (5) had demonstrated that as the Me_{50} dose of the spasmogen (quantity of methacholine chloride, the response to which is reduced by the antispasmodic to 50% of that to a preantispasmodic dose) increases, its antispasmodic equivalent increases, indicating a competitive inhibition mechanism. This work pointed out further that the equivalence of spasmogen and antispasmodic which obtains in antimotility testing differs from that which obtains in urinary retention and antisalivary testing.

This difference is significant in that the mean antispasmodic value (Me_{50}) for gastrointestinal motility did not fall within the confidence limits of the mean values for either urinary bladder or salivary activity. Such significance is indicative of the fact that the receptor sites for these different parameters are, in fact, different with regard to their respective affinities for the antispasmodic.

The Me_{50} is a dose of methacholine the response to which is deduced by the antispasmodic to 50% of the response to a previous rose of methacholine (3). The lower the ratio values (Tables I–IV) obtained from pairs of Me_{50} values, the lower the side-effect liability

Table IV—Ratios (Side Effect/Spasmolytic) Obtained in the Same and Different Determinations

	Same[a]		Different (Same Animal)[b]	Different (Different Animal)[b]	
Atropine, 30 mcg./kg.					
Urinary/GI (motility)	1.73		1.49, 2.69, 1.42	1.43	1.75
	1.77				
	1.58				
	1.44				
	2.24				
Salivary/GI (motility)	3.38		2.87, 2.92, 3.58, 2.83	3.30	2.96
	2.76				
	2.36				
	2.56				
Isopropamide, 8 mcg./kg.					
Urinary/GI (motility)	2.74		2.12, 1.41, 3.07, 2.54	2.78	2.46
Salivary/GI (motility)	3.28		3.40, 3.70, 4.17, 3.21	3.62	3.71
Glycopyrrolate, 8 mcg./kg.					
Urinary/GI (motility)	1.48	1.29	1.35, 1.70	1.61	
	1.34	1.43			
	1.41	1.53			
	1.38				
Salivary/GI (motility)	1.8	1.93	1.80, 2.35	2.08	
	1.98	1.96			
	1.96	1.70			
	1.78				
Poldine, 12 mcg./kg.					
Urinary/GI (motility)	0.90		1.17, 1.23	1.42	
Salivary/GI (motility)	3.60		2.61, 1.92	2.48	
Propantheline, 30 mcg./kg.					
Urinary/GI (motility)			1.87	1.75	
Salivary/GI (motility)			3.14	3.06	

[a] Typical ratios of individual values. [b] Ratios of mean values.

with respect to spasmolytic effectiveness and the greater the usefulness of the compounds undergoing testing.

No appreciable mydriatic effect was seen with any of the compounds tested in the doses employed except in the case of atropine where the response was notable. Since it was not possible with the means employed to measure pupil change with sufficient accuracy for quantitation of the response, a new procedure for the evaluation of the effect of a compound on the ciliary muscle itself is under investigation. Even though accurately measured, pupil size is not an infallible index to lens thickness (or blurring of vision) since the two are not always affected in the same way by a single agent.

All compounds tested tended to diminish gastric acidity and volume increases elicited by methacholine chloride. The data of these parameters were difficult to quantitate, however, and other means of recording these changes are under investigation.

With respect to tachycardia, atropine caused the greatest increase in rate, glycopyrrolate the least of those tested.

DISCUSSION

That such a combination assay as is presented is feasible is attested by the fact that it is possible to obtain gastrointestinal motility, urinary bladder activity, and salivary secretion Me_{50} values with the same dosage range of antispasmodic (Tables I and IV), in spite of the fact that in general there is a difference in the affinity of the various receptors involved for the antispasmodic. (In decreasing order this would be salivary, urinary, and gastrointestinal motility.)

An attempt has been made in this phase of the continuing study to determine whether the entire drug-response picture of such anticholinergic agents can be obtained in a single procedure or whether it must be obtained piecemeal in several procedures.

Side effect/activity ratios derived from results obtained in piecemeal manner are not comparable with those derived from results obtained in the same determination and animal unless the piecemeal ratios are representative of the *means* of the collected data rather than the data themselves (Tables I–IV).

It would appear that sensitivity changes from one determination to another involve all parameters to much the same extent. Side effect/activity ratios obtained in the same determination are therefore more meaningful than such ratios obtained in a number of different determinations.

SUMMARY

A biological assay for the evaluation of antispasmodic activity in the gastrointestinal tract has been expanded to include the evaluation of antisecretory activity (salivary and gastric), urinary retention, and pupillary change effects together with heart rate, blood pressure, and respiratory alterations.

The results (ratios) obtained in such a multiparameter assay are likely more valid than those obtained from the means of values provided in single-parameter determinations. This is indicated by the great variability of the "individual" results of single-parameter determinations depending upon which individual values are cross-paired and the fact that only the ratios of mean values are at all comparable to the multiparameter results.

The method presented provides more information per antispasmodic compound with greater economy of time, effort, and expense than other methods for the evaluation of antispasmodic activity. The information provided is also more useful since it specifies the effectiveness of the agent involved in relation to its side-effect propensity.

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Synthesis and Biological Activity of the Ketals of Digitoxigenone and Digoxigenone and Some Acetals of Digitoxigenin and Digoxigenin

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Abstract □ The preparation of the 3,3-ethylenedioxy derivatives of digitoxigenone and 3-dehydrodigoxigenin; and the 3β-1′-(6′-*O*-acetyl-2′,3′,4′-trideoxyaldohexopyranosyl) derivatives of digitoxigenin and digoxigenin have been described. Their pharmacological activities have been determined.

Keyphrases □ Digitoxigenone, digoxigenone ketals—synthesis □ Digitoxigenin, digoxigenin acetals—synthesis □ Pharmacological screening—cardiac aglycone derivatives □ IR spectrophotometry—structure

The minimal essential structural requirements for maximum cardiotonic activity of the cardiac glycosides is present in the genin digitoxigenin. Although the sugar residues at C-3 play a secondary nonessential role in the biological activities of the cardiac glycosides, their contribution to physical, as well as other properties, may be quite significant. For maximum activity, the hydroxyl group at C-3 should have the β-(axial) configuration that is present in many naturally occurring cardiac glycosides (1). An appreciable loss in activity is found in the α-(equatorial) epimers, especially 3-epidigoxigenin, which is almost devoid of activity (2). An intact glycosidic linkage at the C-3 hydroxyl group is no bar to the biological activity of the cardiac glycosides. This is because they exert a positive and powerful effect upon certain sodium- and potassium-activated ATPases in *in vitro* studies (3). One might thus postulate that the unshared pairs of electrons on the oxygen function at C-3 are one of the essential parameters for activity. It thus would be of interest, from the cardiotonic activity point of view, to test the activity of structurally related derivatives of the cardiac aglycones possessing an oxygen function at both the β- and the α-configurations at C-3. A cyclic ketal of the C-3 keto genins would provide the desired test compound. To the authors' knowledge, such a semisynthetic aglycone derivative has not been prepared. The authors therefore have prepared, for biological testing, the ethylene ketals of digitoxigenone and digoxigenone.

Recently it has been reported (4) that the completely deoxygenated tetrahydropyranyl derivatives of some cardiac aglycones are less active than the parent aglycones, and that deoxygenation in the sugar component also leads to decreased potency. The partially deoxygenated tetrahydropyranyl derivatives, preparation of which is described in this communication, are of considerable interest because they represent a stage between the significantly active monoglucoside (5) derivatives and the less active completely deoxygenated ones. These derivatives of digitoxigenin and digoxigenin were prepared from 2-hydroxymethyl-2,3-dihydro-4*H*-pyran (V), a racemic dideoxyglucal.

The ethylene ketals were prepared primarily by the method described by Dean and Christiansen (6) (Scheme I).

To gain some experience with the Peterson and Gisvold method for preparing tetrahydropyranyl derivatives (7), cholesterol was used as a model compound (Scheme II). All attempts to condense compound V with cholesterol were unsuccessful. Intramolecular reaction of the primary alcohol function with the enol ether double bond (8), or intermolecular condensation to produce the polymer (VII), are possible explanations. These condensations would compete with the acid-catalyzed addition of cholesterol to the enol ether double bond. This view is supported by the authors' finding that conversion of V to the acetate (VIII) circumvents the difficulty.

EXPERIMENTAL

Digitoxigenin—Two grams of digitoxin was hydrolyzed by the method of Yamada (2). The digitoxigenin thus obtained was recrystallized twice from ethyl acetate yielding 740 mg. (73%) of digitoxigenin, m.p. 251–253°, reported (9) 253–255°.

Digitoxigenone (I)—*Method a: Oxidation Using Jones Reagent* (2)—Digitoxigenin 280 mg., dissolved in 38 ml. of acetone (previously distilled with potassium permanganate), was cooled to 10°. Twenty-five hundredths milliliter of Jones reagent (10) was added rapidly while the mixture was stirred vigorously. After 2 min. the reaction mixture was diluted with 40 ml. of water. The product was extracted with ethyl acetate, the extract was washed successively with 5%