SHORT COMMUNICATION

ANTAGONISM OF ANTHRACENE - 9 - CARBOXYLIC ACID AS SCREENING TEST FOR MUSCLE RELAXANTS

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Summary: Veratrinic response produced by anthracene–9–carboxylic acid in mice has been used as a model for studying effect of variety of drugs which induce muscle relaxation. None of the drugs listed as general depressants, central muscle relaxants, neuromuscular blocking agents and anti-convulsants were effective even at doses causing 100% loss of righting reflex. Local anaesthetics/direct acting muscle relaxants were effective. This simple model employed in conjuction with Straub's tail test can differentiate various categories of muscle relaxants and their mechanism of action.

Key words: anthracene–9–carboxylic acid, antagonism, veratrinic response, muscle relaxants

INTRODUCTION

Several synthetic phenanthrene-9-carboxylic acids (8) and substituted benzoic acid derivatives (6) have been reported to produce veratrine like response (an extensor spasm of hind legs triggered by exertion) similar to that produced by veratrum alkaloids (3). Among the various compounds tested for myotonic effect; 3-chloro-2,5,6-trimethyl benzoic acid was found most effective (ED_{50}-4.2. *mg/kg*), but-was difficult to synthesize in pure form and not available commercially (5); whereas anthracene-9-carboxylic acid though not very active, was commercially available.

In the present study, several categories of skeletal muscle relaxants have been studied for their ability to antagonise the veratrine like action of anthracene-9-carboxylic acid, a model for screening compounds useful as muscle relaxants and for treating Myotonia congenita.

MATERIALS AND METHODS

Myotonic action of anthrancene-9-carboxylic acid (ACA): Forty albino mice of same sex & strain (20-25 g) in 10 groups of 4 each, were given graded doses of ACA intraperitoneally and were tested by dropping by tail to a table from the height of 15 cm. at 5 min. interval for 15 min. (3 observation). A positive response consists of hind limb extension spasm and a dragging gait. Myotonic ED_{50} & ED_{100} were calculated by log-probit method of Miller and Tainter (4).

Antagonism of myotonic action of ACA : Albino mice in group of 10 each were treated with the candidate compound and after 30 min., both the control and treated groups were challenged intraperitoneally with ED_{100} dose of ACA, animals tested for the response as described above. The drug was judged effective if at a particular dose it protected the animals against veratrinic action of ACA; PD_{50} and standard error calculated by log-probit method.

Drugs : Drugs and ACA were administered i.p. in 0.5% carboxymethylcellulose ; volumes did not exceed 1% body weight. Test compounds: pentobarbital sodium (Abbott Labs), mephenesin (BDH), meprobamate (Therapeutic Pharmaceuticals), diazapam (Ranbaxy Labs), gallamine triethiodide (May & Baker), succinylcholine chloride (Glaxo Labs), procainamide hydrochloride (Sarabhai Chemicals), quinidine sulphate and (±)-tubocurarine (Burroughs Wellcome & Co.), quinine sulphate (Govt. Alkaloid Factory), diphenyl hydantoin (Parke-Davis & Co.), dantrolene sodium (Norwich Pharmcal Co., U.S.A.), anthracene-9-carboxylic acid (Aldrich Chemical Inc., U.S.A.).

RESULTS

Myotonic ED_{50} & ED_{100} of ACA was found to be 11.5 *mg* and 20 *mg/kg* respectively (Moffett and Tang (6) have reported ED_{50} 8.0 *mg/kg* for this compound).

A total of 12 drugs of different categories were tested in this method and judged effective (+) or ineffective (—), see method.

None of the drugs classified as general depressants, central muscle relaxants, neuromuscular blocking agents and anti-convulsants were effective even at doses causing 100% loss of righting reflex; doses above this were not tried. Only drugs classified as local anaesthetics/direct acting muscle relaxants were effective; most effective being dantrolene sodium, a new muscle relaxant having action on the muscles similar to procaine hydrochloride (2) and quinine, a standard treatment for Myotonia congenita (Table I).

TABLE I: Effect of drugs on veratrinic response of anthracene-9-carboxylic acid.

Drug name	*Effect*	PD_{50} ±*SE(mg/kg)*
General depressants		
pentobarbital sodium	—	
Central muscle relaxants		
mephenesin	—	
meprobamate	—	
diazapam	—	
Neuromuscular blocking agents		
(±)—tubocurarine	—	
gallamine triethiodide	—	
succinylcholine Cl	—	
Anti-convulsants		
diphenylhydantoin sodium	—	
Direct acting/Local anaesthetics		
procainamide hydrochloride	+	165.75±14.50
quinidine sulphate	+	54.75± 4.85
quinine sulphate	+	25.30± 3.45
dantrolene sodium	+	19.50± 1.65

DISCUSSION

Number of tests such as strychnine and leptazole convulsion test and Straub's tail test are available for screening muscle relaxants. The main problem with the former is that the drugs show their effects rather at high doses and the mortality is very high (7); whereas in the latter, variety of compounds are active (1). In the present test, only local anaesthetics/direct acting muscle relaxants are effective. If used in conjunction with Straub's test, it can differentiate various categories of muscle relaxants and give some idea about their mechanisms of action. Further the mortality is almost nil and the animals can be re-employed after suitable intervals.

CONCLUSION

This test provides a quick simple means for assessing direct acting muscle relaxants and compounds useful in Myotonia congenita.

REFERENCES

1. Ellis, K. O. and J. F. Carpenter. A comparative study of dantrolene sodium and other skeletal muscle relaxants with Straub tail muose. *Neuropharmacology,* **13**: 211-214, 1974.
2. Ellis, K.O. and J.F. Carpenter. Mechanism of control of skeletal muscle contraction by dantrolene sodium. *Arch. Phys. Med. Rehabil.,* **55**: 362-369, 1974.
3. Krayer, O. and E. H. Acheson. Pharmacology of Veratrum alkaloids. *Physiol. Rev.,* **26**: 383-446, 1946.
4. Miller, L.C. and M.L. Tainter. Estimation of ED_{50} and its error by means of log-probit graph paper. *Proc. Sec. Expt. Biol.,* **57**: 261, 1944.
5. Moffett, R.B. *Personal communications,* 1971.
6. Moffett, R. B. and A.H. Tang. Skeletal muscle stimulants: substituted benzoic acid. *J. Med. Chem.,* **11**: 1020-1022, 1968.
7. Roszkowski, A.P. Pharmacological comparison of therapeutically useful centrally acting relaxants. *J. Pharmac. Expt. Ther.,* **129**: 75-81, 1969.
8. Smith, R.C. Studies of Phenanthrene derivatives IV. A veratrine like action on skeletal muscles of certain 9-substituted products of phenanthrene. *J. Pharmac. Expt. Ther.,* **59**: 87, 1935.