THE TOXICITY AND MUSCULAR EFFECT OF d-TUBOCURARINE COMBINED WITH β-ERYTHROIDINE, MYANESIN OR EVIPAL*

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It has been shown that curare can relieve muscular spasm of varied origin (1–4). The narrow margin between effective doses and those causing systemic effects seriously limits the usefulness of the drug (5). The experiments presented in this paper were carried out to discover whether it would be possible by the simultaneous administration of other agents to increase the margin of safety and usefulness of the mixture.

The drugs administered jointly with d-tubocurarine were β-erythroidine, myanesin and evipal. β-Erythroidine was chosen because it has a similar action on the myoneural junction as d-tubocurarine, but a different action on the central nervous system (5, 6). Because of the possibility of influencing the peripheral action of d-tubocurarine by the simultaneous use of central depressants, myanesin and evipal were used. Myanesin does not influence the myoneural mechanisms in tolerated doses, but has a depressant effect on the spinal cord (7, 8). With larger doses an ascending depression of the motor pathways of the central nervous system is obtained. Certain pharmacological and clinical experiments indicate that myanesin may have a selective depressant action on the nuclei of the midbrain (9, 10). It has been used clinically as a substitute for curare during anesthesia (11). Evipal is a typical short acting barbiturate causing a descending paralysis of the central nervous system. It has been frequently used in conjunction with curare during anesthesia.

EXPERIMENTAL TECHNIQUE. All experiments were carried out on white, male mice weighing 14 to 20 grams. The drugs were injected intraperitoneally according to the body weight of the animals.

The influence of drugs or mixtures of drugs on muscle power was estimated by the rotating cylinder technique described by Young and Lewis (12) for the mouse assay of insulin and applied by Skinner and Young (13) for the assay of curare. The animals were placed into the rotating cylinder immediately after injection and mice falling away from the cylinder during 20 minutes were considered as reactors. With lethal doses, the number of deaths occurring 24 hours after injection was counted.

Groups of 10 to 40 mice were injected with graded doses of the drugs or mixtures of drugs and the mean effective or lethal dose and its standard error evaluated according to the method of Miller and Tainter (14). The slope of the dosage mortality line was calculated according to Lichtfield and Fertig's (15) formula.

RESULTS AND DISCUSSION. Table I gives the mean effective and lethal doses, their standard errors, and the slopes of the dosage-effect lines of the drugs when administered individually.

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It is of interest to note that d-tubocurarine had the steepest dosage-effect line of the four drugs. The slope of the dosage-mortality line of d-tubocurarine was, however, quite flat, indicating that the response of the animals to muscular effects of d-tubocurarine was much more uniform than their response to the lethal action of the drug.

The experimental design for examining the joint action of drugs was similar to that proposed by Bliss (16). Mixtures of d-tubocurarine with one of the other drugs were prepared in proportions of 1:3, 2:2 and 3:1 in terms of their mean effective (ED_{50}) and mean lethal doses (LD_{50}). Graded doses of each mixture were injected to groups of mice and dosage-effect curves constructed. The mean doses of the various mixtures were found graphically and expressed in terms of d-tubocurarine.

The combined action of two drugs administered jointly may be classified in several groups: (1) Independent action occurs when the action of one drug is not

TABLE I

Mean effective and mean lethal doses of d-tubocurarine, β-erythroidine, myanesin and evipal on intraperitoneal administration to male white mice

	$LD^*_{50} \pm SE^*$	b*	$ED^*_{50} \pm SE$	b
d-Tubocurarine	0.5 ± 0.034	9.0	0.2 ± 0.009	18.0
β-Erythroidine	24.0 ± 0.93	17.3	13.8 ± 0.96	13.1
Myanesin	600.0 ± 22.4	20.7	92.0 ± 6.7	10.7
Evipal	280.0 ± 20.4	12.5	28.0 ± 1.8	14.4

LD_{50} Mean lethal dose in mg./kg.
ED_{50} Mean effective dose in mg./kg.
SE Standard error.
b Slope.

markedly influenced by the presence of the other. When the curves for the two constituents differ in slope, one may expect an abrupt break in the dosage-effect curve of the mixture (16). The two rectilinear segments above and below the break would be expected to have a similar slope as the original constituents. (2) Additive action may be complete when the combined administration of two drugs in complementing proportions of their equitoxic doses causes a similar effect as an equitoxic dose of either constituent administered alone. The two constituents behave as if they were the same substance (substitutive addition of Loewe, (17)). Incomplete addition occurs when the combined effects of the two drugs is greater than that of each constituent administered alone, but smaller than that expected on the basis of arithmetical summation. This type of synergism has been called hetero addition by Loewe (17). (3) Potentiation occurs when the combined effect of the mixture is greater than would be expected from simple addition.

The type of synergism between the action of two drugs can be illustrated graphically by plotting equitoxic doses of various mixtures of the drugs in terms of the more potent constituent against the concentration of the other constituents of the mixture. In Figure 1. the line OA represents the mean lethal dose of curare in the absence of other drugs and the line OB the mean lethal doses of the other drugs when given alone. If an effect produced by the combination of two drugs lies on the line AB, the drugs have a complete additive action. If the effect of the combined action of two drugs is represented by points inside the triangle AOB, potentiation is taking place, and if it is represented by points above the line AB incomplete addition is spoken of. In the case of independent and different action the points would lie near a line drawn through point A parallel to OB.

The mean lethal and mean effective doses and their standard errors expressed in terms of d-tubocurarine are given in Table II. The slopes of the individual dosage-effect lines and the deviation of the observed mean dose expressed as a percentage of the dose expected to give an additive effect is also given. The results are also illustrated in Figure I.

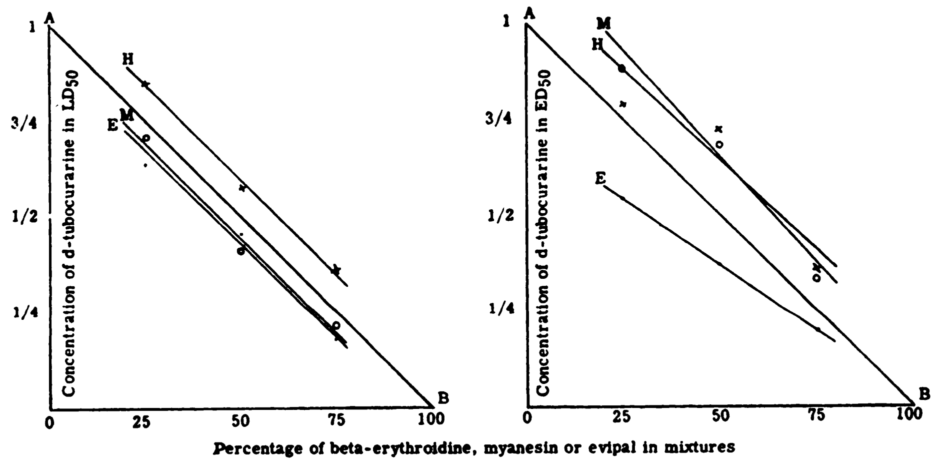

FIG. 1. The mean effective and mean lethal doses of mixtures of d-tubocurarine with β-erythroidine, myanesin or evipal, plotted in terms of d-tubocurarine against the concentration of the other constituent in the mixtures.

When lethal doses were given, the combined effect of d-tubocurarine and β-erythroidine or myanesin was potentiated. The joint action of d-tubocurarine and evipal was incompletely additive. The effect obtained with the three mixtures of each pair of drugs appeared to be largely independent from the ratio of one component to the other. When the LD_{50} doses of the mixtures were plotted against their percentage compositions straight lines parallel with summation line AB were obtained.

When the effect of the combinations was judged by the muscular weakness as measured by the inability of the animals to maintain themselves on the rotating cylinder different results were obtained. The joint effectiveness of d-tubocuarine and β-erythroidine was potentiated to a greater extent than their lethal action. The ED_{50} doses of the mixtures plotted against their percentage composition again gave a straight line which was, however, flatter than the summation line

AB indicating that over the range of combinations examined potentiation increased in proportion to the d-tubocurarine contents.

d-Tubocurarine administered with myanesin or evipal were less effective than would have been expected on the basis of simple summation of effects. The ED_{50} dose plotted against their percentage composition gave curves showing an upward convexity, indicating that summation was least when the mixtures consisted of equal parts of each constituent. This relation was particularly marked in the case of evipal.

TABLE II

Mean effective and lethal doses after combined administration of d-tubocurarine and β-erythroidine, myanesin or evipal on intraperitoneal administration to male white mice

Doses in mg. per kg. body weight expressed in terms of d-tubocurarine

RATIO OF DRUGS	$LD^*_{50} \pm SE^*$	b*	D*	$ED^*_{50} \pm SE$	b	D
1 T* + 3 E*	0.096 ± 0.006	10.4	−23	0.037 ± 0.003	6.5	−26
2 T + 2 E	0.222 ± 0.011	15.6	−11	0.071 ± 0.005	5.5	−29
3 T + 1 E	0.315 ± 0.022	12.6	−16	0.107 ± 0.007	8.2	−29
1 T + 3 M*	0.104 ± 0.0048	23.4	−17	0.065 ± 0.006	9.2	+30
2 T + 2 M	0.205 ± 0.014	22.5	−18	0.134 ± 0.008	14.5	+34
3 T + 1 M	0.35 ± 0.014	22.3	−7	0.173 ± 0.016	7.9	+15
1 T + 3 H*	0.172 ± 0.014	9.4	+38	0.071 ± 0.009	5.6	+42
2 T + 2 H	0.282 ± 0.014	14.0	+13	0.143 ± 0.02	3.1	+43
3 T + 1 H	0.42 ± 0.014	16.6	+12	0.157 ± 0.016	5.2	+5

T d-Tubocurarine.
E β-Erythroidine.
M Myanesin.
H Evipal.
LD_{50} Mean Lethal Dose in mg./kg.
ED_{50} Mean Effective Dose in mg./kg.
SE Standard error.
b Slope of the dosage-effect line.
D Percent deviation from dose giving additive effect expressed in terms of d-tubocurarine.

In order to ascertain whether the combined administration of d-tubocurarine and β-erythroidine would be more advantageous and safer than d-tubocurarine given alone, it was necessary to take into consideration not only the potentiated effect, but also the relative safety of such a combination. The so called "therapeutic index" of a drug or combination of drugs is often given as a ratio of the mean lethal dose to the mean effective dose. Expressed in this way the values for d-tubocurarine and β-erythroidine administered alone were 2.5 and 1.74. The ratios of the mean lethal to the mean effective dose of the three mixtures of these drugs gave somewhat higher values, namely 2.6, 3.1 and 2.9. Different results were, however, obtained when due attention was given to the slopes of the dosage-mortality and dosage-effect lines. Foster (18), taking this into account, proposed the use of the standard safety margin which gives the percentage above the surely effective dose (ED_{99}) at which an occasional death will occur (LD_1) according to the formula $\left(\frac{LD_1}{ED_{99}} - 1\right) 100$. d-Tubocurarine given alone had a standard safety margin of 3%. Mixtures of d-tubocurarine and β-erythroidine had no margin of safety. It would, therefore, appear that the joint administration of the two drugs would be less safe than an equally effective dose of d-tubocurarine given alone. It is, however, possible that some of the side effects of both drugs may cancel each other out on joint administration in man. There is no evidence of this in mice. All animals receiving a mean effective dose of d-tubocurarine showed little side effects apart from a partial loss of muscular strength and coordination as evidenced by their inability to maintain themselves on the cylinder. Animals receiving a mean effective dose of d-tubocurarine and β-erythroidine on the other hand appeared somewhat hyperexcitable, and 3 out of 40 of those injected with the mixture containing the drugs in the proportion of 2:2 developed tonic convulsions and died.

The joint lethal action of d-tubocurarine and myanesin was potentiated, but there was only incomplete summation of the effects as judged by the ability of the animals to maintain themselves on the rotating cylinder. These observations are in agreement with the findings (8) that myanesin does not possess curare-like action in the small doses affecting voluntary muscles. The slight degree of synergism observed between d-tubocurarine and myanesin differs from the potentiation apparent after joint administration of myanesin and evipal (8).

In view of the frequent use of d-tubocurarine during barbiturate anesthesia the joint effect of the two drugs is of interest. Under the conditions of these experiments the joint effect of d-tubocurarine and evipal resulted in incompletely additive action. The dosage-effect lines of the combinations were very flat and there was no standard margin of safety between lethal and effective doses. Although excellent results have been obtained by the use of curare during anesthesia, some anesthetists believe that the procedure is risky and decreases the safety of anesthesia. The experiments described in this paper appear to support this contention.

The results of this investigation indicate that there is little likelihood of increasing the usefulness of d-tubocurarine by the simultaneous administration of other drugs. The margin of safety of d-tubocurarine administered alone is small enough and joint administration of three other drugs with widely differing modes of action reduced this margin still further. In the opinion of the authors the real advance in the treatment of spastic and dystonic states awaits the discovery of drugs possessing a selective depressant action on specified levels of the central nervous system.

SUMMARY

d-Tubocurarine and β-erythroidine administered jointly show potentiation in effective and lethal doses. The margin of safety of the mixture of the drugs is

smaller than that of d-tubocurarine administered alone. The combined administration of d-tubocurarine and myanesin showed slight potentiation in lethal doses and incomplete additive action in effective doses. d-Tubocurarine and evipal showed incomplete additive action in both effective and lethal doses. The significance of the findings is discussed.

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