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Quantitative Examination of the Interaction of Competitive Neuromuscular Blocking Agents on the Indirectly Elicited Muscle Twitch

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Gallamine, metocurine, pancuronium, and *d*-tubocurarine were compared when given alone and in combination with isolated guinea pig nerve-lumbrical preparations stimulated via the nerve. The experimental design was set up to control the effects of variation among preparations, order of administration, and time of administration (*i.e.*, fresh *vs.* older preparation). The result was an assay able to measure potentiation with a coefficient of variation of 3%. A format for a graphic presentation to summarize such results is presented and discussed. Two combinations, gallamine plus *d*-tubocurarine and gallamine plus pancuronium, showed no sign of an interaction beyond that to be expected from a simple competitive interaction. Two others, metocurine plus pancuronium and gallamine plus metocurine, showed about a twofold greater potency when combined than would have been expected. The last two sets, pancuronium plus *d*-tubocurarine and metocurine plus *d*-tubocurarine, showed a slight degree of potentiation. These studies demonstrate that the deviation from simple additivity seen *in vivo* persists when examined in a system free from artifacts associated with uptake and distribution in the whole organism. (Key words: Neuromuscular relaxants: *d*-tubocurarine; gallamine; metocurine; pancuronium. Interaction: neuromuscular relaxants.)

DRUGS like *d*-tubocurarine show kinetic behavior in excellent agreement with a competitive interaction with a test agonist. In particular, when the experiments are summarized in a Schild plot, a linear relationship with the unit slope expected from a competitive interaction is obtained.[1,2] If indeed these competitive kinetics imply a competitive mechanism, then two agents when mixed should interact in an additive manner.‡ For example, if concentration C_A of drug A and C_B of drug B produce some response, then that same response should be produced by a mixture containing drugs A and B at concentrations $C_A/2$ and $C_B/2$. Riker and Wescoe[3] showed such an additive effect of *d*-tubocurarine and gallamine in cat tibialis anterior. However, Wong,[4] using LD_{50} in mice, head-drop in rabbit, or contraction of the gastrocnemius muscle in rabbits, and Ghoneim *et al.*,[5] using patients, found that a mixture of *d*-tubocurarine and gallamine resulted in a block that was greater than that which would be expected by simple addition. Lebowitz *et al.*,[6] in patients, showed a greater than additive effect with pancuronium–metocurine and pancuronium–*d*-tubocurarine combinations but not with a metocurine–*d*-tubocurarine combination. Schuh,[7] on the other hand, described only an additive interaction with pancuronium–*d*-tubocurarine, pancuronium–gallamine, or *d*-tubocurarine–gallamine combinations in patients. All of the above experiments were done *in vivo* where extraneous factors such as pharmacokinetic asymmetries could have caused the observed departures from competitivity. This interpretation is reinforced by the lack of consistency among *in vivo* studies.

In a system *in vitro*, an isolated rat phrenic nerve–diaphragm preparation, Pollard and Jones[8] have reported that a mixture of pancuronium and *d*-tubocurarine produced more than an additive effect. However, the drugs were given only 5 min for equilibration, so once again one cannot be sure that the observed aberrations did not reflect differences in concentrations at the site of action rather than properties of the end-plate. Another complication in nerve–muscle preparations is that the nerve has been cut, so presumably the process of degeneration will begin at some point in time. Thus, it is possible, at least, that the margin of safety may become lower later in the experiment (Paton and Waud[9] thought they could detect such a change) and thus blur interpretation of results. (This argument is based on the observation that about 80% of receptors must be blocked before neuromuscular transmission begins to fail. This implies that the nerve puts out far more transmitter than is needed barely to trigger the muscle fiber, *i.e.*, there is a large margin of safety. However, if the nerve has been cut, the transmitter stores presumably will begin to fall sooner or later. Thus, with time, the margin of safety will fall and sensitivity to neuromuscular blocking agents might be expected to increase.)

We decided, therefore, to attempt a "state-of-the-art" biologic assay to control as many variables as possible to demonstrate that, in a tightly controlled experiment, either the drugs were additive or that there

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‡ We shall subdivide interactions into two subclasses: 1) additivity, implying working together to the extent expected from two drugs interacting competitively with the same receptor, and 2) potentiation implying an effect greater than additive.

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was potentiation that could not be explained by a recognized extraneous factor.

Methods

The experiments were done in isolated guinea pig nerve–lumbrical muscle preparations. The tissue was mounted for isometric twitch recording in an isolated organ bath at 37° C and in Krebs' solution of composition (mM) Na^+ 138, K^+ 5.9, Cl^- 123, Ca^{++} 2.5, Mg^{++} 1.22, $H_2PO_4^-$ 1.2, SO_4^{--} 1.22, HCO_3^- 25, plus glucose 2.08 g/l, and bubbled with 95° oxygen/5% carbon dioxide. The muscle was stretched to the initial tension, which produced maximal developed tension and then left about half an hour to reach a steady level of twitch response. The nerve was stimulated supramaximally with 0.1-ms shocks every 10 s. At this point, the first of three cumulative dose response curves was determined. The drug (or combination of two drugs) was added stepwise and at each level left in contact with the muscle until no further detectable change in twitch height occurred for a minimum of 5 min. (This equilibration time generally lasted about 30–60 min. The bath solution was refreshed every half hour.) After the highest concentrations to be tested had been reached, the preparation was washed out for 15–30 min and the next curve determined.

Statistical Analysis

The experiments involved a very sophisticated statistical analysis, essentially a so-called split plot[10] factorial analysis of a nonlinear regression. We examined four drugs, *d*-tubocurarine, pancuronium, gallamine, and metocurine in all six possible pairings. Each pairing was analyzed as a unit in an assay involving six muscle preparations. In each muscle there were three dose–response curves. One involved drug A, one drug B, and one the combination (C). The order of these applications was varied systematically among the six muscles to give the following sets: ABC, BCA, CAB and CBA, ACB, BAC where the second set of three is the first set in the reversed direction. This design not only ensures that each of A, B, and C comes at each point in time but also allows one to determine statistically whether the "permutation" of drug application (ABC *vs.* BCA *vs.* CAB) or the "direction" (ABC *vs.* CBA) affected the result. It is similarly possible to detect (and, more importantly, eliminate statistically) the effect of a temporal change in sensitivity. Thus by comparing the ED_{50} obtained in all the first curves with all the second and all the third, one gets contributions with 2As, 2Bs, and 2Cs in each (*i.e.*, balanced regarding drug) so any difference is due to "time."

The actual calculations are complicated by the fact that a dose–response curve is not a straight line. We did not wish to use the common short-cut of fitting a straight line to the steep part of the curve but rather chose the more unwieldy but more rigorous approach of fitting a sigmoid curve. To this end we chose the "logistic" function:

$$y = 1 - x^S/(x^S + 1) \quad (1)$$

This is a curve that is 1 when x is zero, approaches zero as x gets large and has a steepness governed by S. (Although the logistic curve can, unlike some other functions one might have used, be viewed as a generalization of the equations describing a drug–receptor reaction, the logistic function was chosen here mainly because of familiarity and computational convenience. There is no reason to believe essentially identical results could not have been obtained with another s-shaped function such as the probit or arctangent).

In equation (1), y represents the normalized twitch response, *i.e.*, the twitch response as a fraction of the value in the absence of drug, while x represents the normalized concentration of antagonist. The natural unit of concentration is the actual concentration divided by the dissociation constant of the drug–receptor reaction.[2] This latter value is not available directly in the sort of experiment being analyzed here. However, if one starts with a competitive model (the null hypothesis in the present study), then the ED_{50} for reduction in the twitch response will be a constant factor times the drug–receptor dissociation constant. On this basis, therefore, we have taken the x in equation (1) to be

$$x = A/ED_{50_A} \quad (2a)$$

$$= B/ED_{50_B} \quad (2b)$$

or

$$= A/ED_{50_A} + B/ED_{50_B} \quad (2c)$$

for the curve in the presence of drug A, drug B, or the combined drugs A and B respectively.

One more level of complexity must now be superimposed. The ED_{50} of equation (2) was multiplied by one or more modifiers to give a product of the form

$$ED_{50} \times (G_i) \times \binom{P}{D} \times (T) \times (C) \quad (3)$$

where the parentheses imply the modifier appears only when appropriate. First, we can indicate the nature of each modifier and then outline how it was used.

The modifier G_i is a factor that accounts for the fact that each guinea pig muscle will be expected to exhibit its own sensitivity. Thus, if guinea pig 3 had a higher margin of safety than number 5, 3 would require more

of all drugs to achieve any given level of effect, so G_3 would be bigger than G_5.

The modifiers P and D behave similarly. If the choice of different permutations (ABC *vs.* BCA, *etc.*) affects the sensitivity, then the permutation parameters will reflect (and allow for) this and be other than unity. Similarly, if "direction" (ABC *vs.* CBA, *etc.*) is a significant factor, this will show up in the D parameter.

The modifier T is very important; it takes care of the effect of time. Thus, if, as expected, the margin of safety drops with time, we can partition this factor into the T modifier and so remove its effects from the analysis of synergism.

Finally we come to the C modifier. This is the focus of the whole study. The parameter C is a factor used to bring the combination curve into line with the other two. Thus, if there is no potentiation, the value of C will be unity, *i.e.*, we don't have to change the ED_{50} of the combination curve to fit the results closely. On the other hand, if there is synergism beyond additivity, *i.e.*, if there is potentiation, the combination curve will be further to the left than would be consistent with the A and B curves, so the parameter C will have to be significantly less than unity to produce a good fit.

We must now address the analysis of variance to show how the modifiers were used. First consider permutation and direction (P and D). We fitted the observations to a series of equations in which modifiers were systematically included or left out. Specifically we used

Fit 1: S, ED_{50_A}, ED_{50_B}, G_2, G_3, G_4, G_5, G_6
Fit 2: S, ED_{50_A}, ED_{50_B}, P_2, P_3, D_2
Fit 3: S, ED_{50_A}, ED_{50_B}, P_2, P_3
Fit 4: S, ED_{50_A}, ED_{50_B}, D_2
Fit 5: S, ED_{50_A}, ED_{50_B},

(With two drugs and six guinea pigs you can specify all ED_{50} values with seven parameters, for example, the ED_{50} of drug A in all six animals and the potency ratio of B relative to A. While this particular choice is most symmetric, and was used if we wanted the actual ED_{50} values ("Fit 9"), we did most of the calculations as indicated above for "Fit 1," using two ED_{50} values for the drugs, and five modifiers. This means one guinea pig must be chosen as a reference and arbitrarily assigned a value of unity, *i.e.*, $G_1 = 1$. For analogous reasons, P_1 and D_1 are also unity.)

Now, in the above set of Fits, number one will be best because it has the most parameters available for adjustment. Thus, the scatter of points about the lines fitted with the eight parameters will give a measure of error. Fit 2 will similarly be better than Fit 5 again because more parameters are available. The difference in scatter about the fitted curves in Fits 2 and 5 will be a measure of whether parameters P and D contribute significantly. Similarly, the increase in error in Fit 2, relative to Fit 1 will give a measure of how much of the variation among pigs is not explained by P and D. Thus, by comparing these last two differences (2 minus 5 relative to 1 minus 2) one can test whether the parameters P and D are significantly different from unity. Analogously, by comparing 2 with 4 and 3 with 5, one can get a measure of the effect of P alone, *etc.*

The preceding analysis of variance represents the "main plot" (c.f. Snedecor and Cochran[10]; the terminology reflects analyses involving agricultural plots of land). The factors of particular interest are in the "subplot" and involve comparisons within animals (rather than between animals as with the G modifiers). The relevant fits are

Fit 6: S, ED_{50_A}, ED_{50_B}, G_2, G_3, G_4, G_5, G_6, C, T_2, T_3
Fit 7: S, ED_{50_A}, ED_{50_B}, G_2, G_3, G_4, G_5, G_6, T_2, T_3
Fit 8: S, ED_{50_A}, ED_{50_B}, G_2, G_3, G_4, G_5, G_6, C
Fit 1: S, ED_{50_A}, ED_{50_B}, G_2, G_3, G_4, G_5, G_6

The effect of time is obtained from the comparisons 6 minus 8 and 7 minus 1, that of synergism (C) from 6 minus 7 and 8 minus 1. (The comparison 6 minus 7 gives a measure of C in the presence of T, 8 minus 1 in its absence. We took the average of these two as our measure of effect. Their difference measures the "interaction" of C and T. Nothing remarkable was seen by way of interaction so the averaging seems justified.)

One last problem remains in the analysis. How do you plot the results? Curves A and B are straightforward; you can plot twitch height against concentration of antagonist, and, to eliminate the arbitrary difference in potency, you can normalize concentrations by dividing by the ED_{50} determined by the statistical fit so all drugs have an ED_{50} of unity. But what do you use for the ED_{50} of a combination? The catch is that such an entity does not exist. The one-dimensional ED_{50} becomes a two-dimensional surface when two drugs are involved. We have decided, therefore, to summarize the results as follows. We had the computer draw a reference curve with slope determined by the fitted value of S from Fit 6 and with an ED_{50} of 1. We used Fit 6 because it gives the best fit to the observations, but, in particular, it allows for the effect of time, which we expected to confuse the issue if not controlled. Next we could normalize all concentrations by dividing by the relevant ED_{50} determined again by the statistical fitting process (after those ED_{50} values had been multiplied by the fitted values of the modifiers used). For the combination curve the sum of the two such normalized concentrations could be used. The observed values then could be plotted at these calculated concentrations. The effect of

all this would be to bring all points over to the reference curve to the extent that this curve represents a good fit to the data. The figures show that this fit is excellent for responses to drugs A and B alone. This close fit is presumably the reward for the effort that went into the experimental design. Now the location of the points from the combination curves depends on the bottom line—"is there more than additivity?" If there is not, that is, if the response of the combination of drugs is simply a reflection of what would be expected on the basis of simple competitive kinetics, then these points will lie over the reference curve like those of the other two sets. If on the other hand, there is an interaction beyond that expected from simple competition, we can show this by plotting the points from Fit 6 but leaving out the parameter C. The effect of this will be to shift the points away from the curve to the extent that C was needed to bring them into line. Finally, we can draw a second curve running through this subgroup of points. Thus, to get an immediate visual summary from one of these "synergism plots," one simply looks at the values for the combination curve (triangles in our figures) and notes whether they lie outside the cloud (representing random variation) formed by the values for drugs A and B given alone (circles and squares). The distance between the two curves similarly will give a measure of deviation from simple additivity. (In borderline cases, of course, one has to go back to the analysis of variance in the usual way to get an objective measure of significance.)

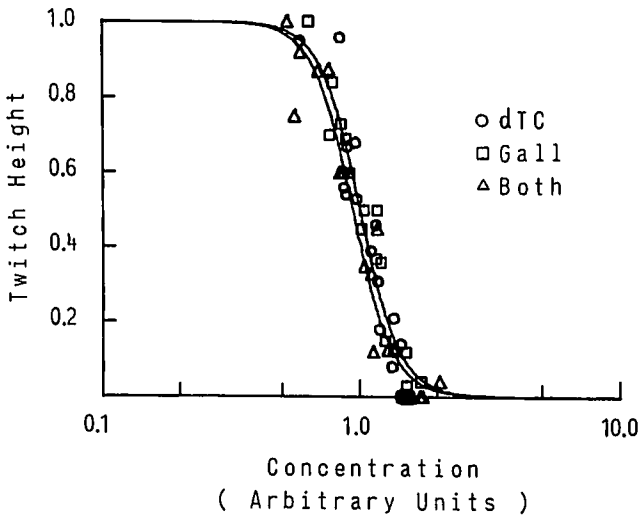

FIG. 1. Graphic summary of the gallamine plus *d*-tubocurarine interaction. Ordinates: twitch responses as fraction of control when no drug was present. Abcissae: normalized concentrations. For drug A (circles, *d*-tubocurarine) and drug B (squares, gallamine) the concentration is calculated as the micromolar concentration applied divided by the effective ED_{50}, which, in turn, was the ED_{50} estimated in Fit 6 (see text) multiplied by the appropriate G and T modifiers. For the A and B combination (triangles) the sum of two such calculated concentrations was used (but the C modifier was omitted). The reference curve was drawn with the steepness parameter determined in Fit 6 and an ED_{50} of unity. The second (left-hand in this figure) curve was drawn with the same slope but with the ED_{50} multiplied by the C factor (0.937 in this case). There is no evidence for potentiation; the drugs are simply additive.

Results

The basic results are shown clearly in figures 1–6. In two cases, gallamine plus *d*-tubocurarine (fig. 1) and gallamine plus pancuronium (fig. 2), there was no evidence of an interaction beyond simple additivity. In two cases at the other extreme, metocurine plus pancuronium (fig. 3) and gallamine plus metocurine (fig. 4), there was a clear-cut potentiation, of the order of twofold. In the remaining two pairs, pancuronium plus *d*-tubocurarine (fig. 5) and metocurine plus *d*-tubocurarine (fig. 6) there was a slight but significant sign of potentiation. Table 1 summarizes the actual estimates of the factor C.

The "permutation" and "direction" factors were without influence in all but one case. On the other hand, "time" was uniformly highly significant. Over all six assays, the average values for the parameters T_2 and T_3 were 0.824 (±0.035 SD) and 0.787 (±0.030 SD), respectively, (referred to a reference T_1 of unity).

While absolute ED_{50} values are not particularly significant, since they reflect idiosyncracies of the test preparation (margin of safety and the like), comparative values, *i.e.*, potency ratios are more meaningful. Table 2 summarizes the values obtained in the present study. For this table we used Fit 9, which gives a direct estimate of the potency ratio. On aesthetic and pharmacologic

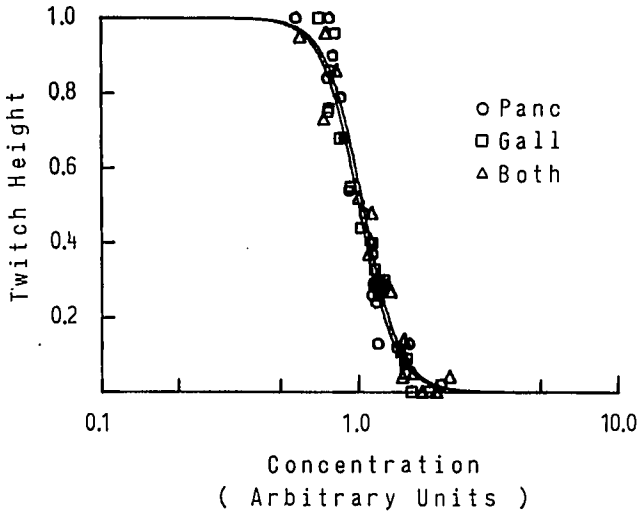

FIG. 2. Graphic summary of the pancuronium plus gallamine interaction. (See figure 1 legend for format.) The drugs are additive.

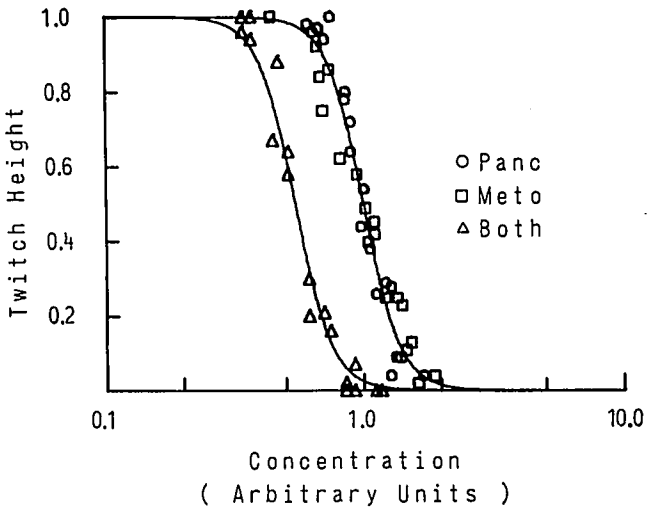

FIG. 3. Graphic summary of the pancuronium plus metocurine interaction. (See figure 1 legend for format.) The combination is clearly more potent than would occur if there were just simple additivity; *i.e.*, the triangles lie well to the left of the other points.

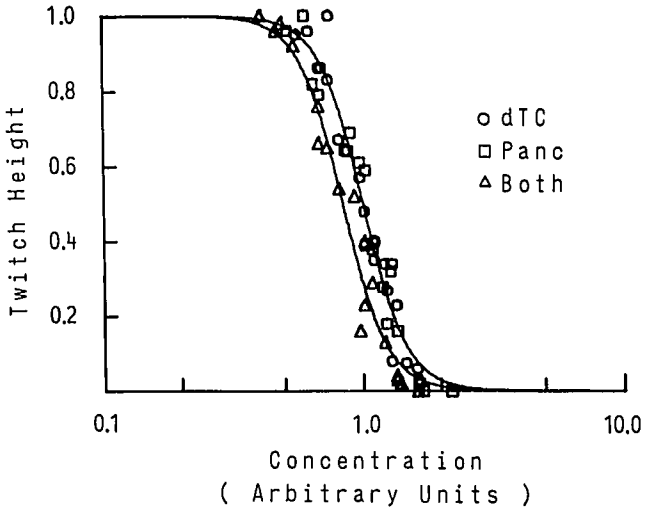

FIG. 5. Graphic summary of the *d*-tubocurarine plus pancuronium interaction. (See figure 1 legend for format.) There is a tendency for the triangles to lie to the left of the other points, *i.e.*, a hint of potentiation.

grounds, the computer was programmed to calculate not the potency ratio but the square root thereof. The reason simply was one of symmetry; there is no natural reason to chose either antagonist A or B as a reference compound. Therefore, we fitted curves with an ED_{50} that was the geometric mean for the two drugs and with a second parameter such that dividing the geometric mean ED_{50} by it gives ED_{50_A} and multiplying by it gives ED_{50_B}. Thus, the square of that parameter corresponds to the potency ratio in the usual sense. The computer yielded a standard error on the original parameter. The formula[11]

$$\text{SE (squared value)} = \sqrt{2 \times \text{unsquared value} \times \text{SE}^2 \text{ (unsquared value)}}$$

was used to convert to the standard error appropriate to the squared value. The corresponding values of the steepness parameter S (from Fit 6) are included in table 2 as well.

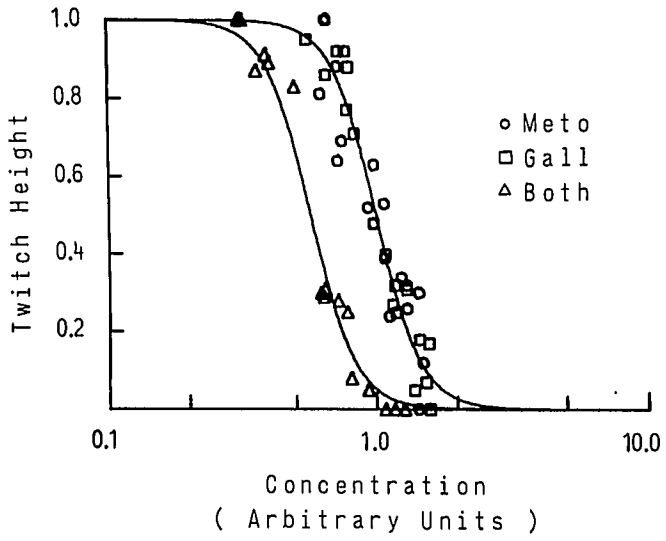

FIG. 4. Graphic summary of the metocurine plus gallamine interaction. (See figure 1 legend for format.) As in figure 3, there is clearly potentiation.

Discussion

The assay produced some results that were expected and some less so. We shall discuss these in turn.

The careful attention to experimental design paid off in a sensitive assay. We were able to detect potentiation factors of a few per cent. The standard error of the C parameter was 0.023 (±0.006 SD) averaged over the six assays to give a coefficient of variation less than 3%.

In the absence of precedent, we did not expect the "permutation" or "direction" effects to be significant and were not surprised. Apparently there is no "memory" of previous history in the system. (The fact that one of the six assays produced a "significant" effect of D and P at the 95% probability level is to be expected. If you repeat a test six times at this significance level, you can expect a "significant" result with a probability of $1 - (0.95)^6 = 0.264$.) On the other hand, as indicated earlier, we did expect an effect of time both because of an impression that the preparation becomes more sensitive as the day wears on and on *a priori* grounds that possible early effects of denervation might appear as an

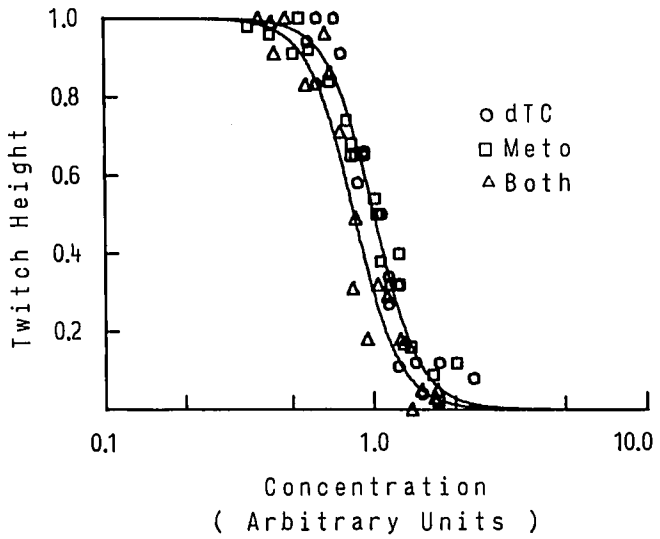

FIG. 6. Graphic summary of the *d*-tubocurarine plus metocurine interaction. (See figure 1 legend for format.) As in the preceding figure, there is a suggestion of potentiation.

increase in sensitivity. The values of T_2 and T_3 of 0.824 and 0.787, respectively, are particularly noteable when compared with the 3% standard error in C, the entity we want to measure. Without elimination of the variation due to time, we would have not been able to pick up fine changes in C.

One might argue that all the fuss over order and time could have been eliminated by simply doing only one curve on each animal. However, experience suggested, and the experimental outcome confirmed, that the split-plot design was a better approach. The variance among animals in the main plot ranged from 2.3-fold to 63-fold greater than the error in the subplot within animals. Thus, an experimental design that allowed the key comparison (potentiation, C) to be made within animals clearly was advantageous.

The potency ratios in table 2 are not of great intrinsic interest by themselves but do provide a valuable cross-check on the internal consistency of all the assays. The six potency ratios all are interconnected inasmuch as, for example, the potency ratio *d*-tubocurarine/gallamine must equal the product of the *d*-tubocurarine/pancuronium and pancuronium/gallamine ratios and also that of the *d*-tubocurarine/metocurine and metocurine/gallamine ratios. We therefore calculated each of these products and divided them by the directly obtained value to get a set of 12 numbers that should, if the assays are coherent, average unity. The average was 1.04, with a standard error of 0.042. Thus, internal consistency is excellent. The low 4% coefficient of variation (0.042/1.04) indicated that this test should be reasonably sensitive. This 4% level of error is similar to

TABLE 1. Summary of Estimates of Potentiation Parameter C

Interaction	C*	SE (C)
Gallamine plus *d*-tubocurarine	0.937	0.033
Gallamine plus pancuronium	1.04	0.024
Metocurine plus pancuronium	0.545†	0.014
Gallamine plus metocurine	0.570†	0.020
Pancuronium plus *d*-tubocurarine	0.837†	0.022
Metocurine plus *d*-tubocurarine	0.845†	0.027

* A value of C = 1 implies simple additivity; one of 0.5 implies the combination was twofold more potent than expected from simple addition.

† The analysis of variance generated a variance ratio for synergism significant at the 99% probability level.

that already noted for C. This indicates the variation between assay units is not grossly out of line with that within.

The significant potentiation observed with some drug combinations was not predictable beforehand. However, there can be little doubt that the phenomenon is real. A glance at either figures 3 or 4 makes that abundantly clear, particularly when the rigor of the experimental design is in mind. The slight shift seen in figures 5 and 6 might be considered of questionable pharmacologic significance in the absence of the strong precedent given by figures 3 and 4 but in that light appears to be a milder version of the same phenomenon.

What then is going on? The present experiments do not allow us to say much of a positive nature. We can safely rule out artifacts such as pharmacokinetic phenomena, inadequate time for equilibration, or inadequate control over temporal drift and order of administration. We then are left with the obvious possibilities: 1) there is a presynaptic contribution confounding the analysis, and/or 2) the postsynaptic interaction is more complicated than a simple competition at a single receptor site. The first step in untangling these two will be to carry

TABLE 2. Summary of Potency Ratios (R) and Steepness Parameters (S)

Drugs	R* (SE)	S† (SE)
d-Tubocurarine/gallamine	5.95 (0.141)	5.55 (0.45)
Pancuronium/gallamine	27.9 (0.475)	6.09 (0.38)
Pancuronium/metocurine	2.27 (0.191)	6.16 (0.41)
Metocurine/gallamine	9.68 (0.489)	5.24 (0.38)
d-Tubocurarine/pancuronium	0.225 (0.012)	5.14 (0.31)
d-Tubocurarine/metocurine	0.552 (0.027)	5.16 (0.38)

* The potency ratio R represents the ratio of the ED_{50} of the second to that of the first drug. Thus a value of 5.95 for "*d*-tubocurarine/gallamine" means that 5.95 times more gallamine than *d*-tubocurarine is needed to produce a given level of block. Values come from Fit 9 (see text).

† The steepness parameter S (c.f. equation 1 in text) is that derived in Fit 6. The average of the 6 values is 5.56 with a standard deviation of 0.46.

TABLE 3. Summary Comparison of Results

	Preparation	Result
Gallamine plus *d*-tubocurarine (additive)*		
(1) Riker and Wescoe[5]	Cat tibialis, *in vivo*	Additive
(2) Wong[4]	Mouse LD_{50}	Potentiation
	Rabbit head drop	Potentiation
	Rabbit gastrocnemius, *in vivo*	Potentiation
(3) Ghoneim *et al.*[5]	Human adductor pollicis	Potentiation
(4) Schuh[7]	Human flexores digitorum	Additive
Gallamine plus pancuronium (additive)*		
(5) Schuh[7]	Human flexores digitorum	Additive
Pancuronium plus *d*-tubocurarine (slight potentiation)*		
(6) Lebowitz *et al.*[6]	Human adductor pollicis	Potentiation
(7) Schuh[7]	Human flexores digitorum	Additive
(8) Pollard and Jones[8]	Rat diaphragm, *in vitro*	Potentiation
Metocurine plus *d*-tubocurarine (slight potentiation)*		
(9) Lebowitz *et al.*[6]	Human adductor pollicis	Additive
Metocurine plus pancuronium (clear potentiation)*		
(10) Lebowitz *et al.*[6]	Human adductor pollicis	Potentiation

* Results in parentheses are those from present study.
No comparison studies were available on the gallamine plus metocurine combination (which showed clear potentiation in the present study).

out an assay analogous to the present one but with depolarization of the end-plate by an agonist as the measured effect. If the potentiation persists in that system, then the phenomenon is postsynaptic, and if the extent is similar in magnitude there will be little reason to look presynaptically. On the other hand, if the potentiation is not seen when depolarization is examined, or if it is reduced in magnitude, the nerve ending comes to the fore.

The pictorial format for summarizing the results is worth comment. Inspection of the figures demonstrates that the format does the job. It is very easy to see the three types of results (by chance we ended up with two examples of each of the interesting cases). There is no doubt in figures 1 and 2 that potentiation is negligible. Similarly, in figures 3 and 4 it is clearly present. Finally, one hardly can be surprised after viewing figures 5 and 6 to learn that the potentiation effect was significant but barely so. Admittedly, the underlying machinations necessary to make the plot require careful thought, however, the end result is a remarkably clear summary.

How do the present results relate to those of previous studies? There are a lot of comparisons to be made, so we have summarized the results in table 3. With the two pairings we found additive, three reports (1), (4) and (5) in table 3, agreed, and two, (2) and (3), did not. With the two combinations we found to produce a slight potentiation, previous studies reported potentiation in two cases, (6) and (8) and additivity in the other two, (7) and (9). Finally, potentiation was reported in the only outside study, (10), examining a pair we found to show marked potentiation. Thus there is general agreement between our results and those reported previously. The earlier studies that are not in line with ours include those in mice and rabbits and two involving combinations that we found produced only a limited degree of potentiation. Whether the rodent studies differ because of species or some *in vivo* (pharmacokinetic?) feature one cannot say. That the other two studies might not be in line is hardly surprising, since the extent of potentiation is so slight. Although this general agreement may seem reassuring, not much emphasis should be placed on the comparisons *per se*. Had they not agreed, the possibility of a species or pharmacokinetic basis for a difference would have been a plausible explanation. Thus, the significance of the present results is not so much that they support or do not support any particular prior study, as much as that they provide a precedent that is as free as possible of any identifiable experimental flaw. In other words, up to now when one encountered an *in vivo* study that reported potentiation, the combination of 1) the ever present possibility that the interaction was pharmacokinetic and not pharmacodynamic with, 2) the absence of a rigorously demonstrated precedent would lead the cautious observer to be somewhat skeptical. The present studies remove the latter basis for reservation and thus give us greater confidence that the potentiation seen *in vivo*, in particular in man, represents a potentiation arising at the neuromuscular junction and not just some chance interaction of secondary effects of the drugs involved.

Finally, it is of interest to comment on the relevance of the potentiation involved to clinical dosing. With metocurine plus pancuronium we observed a twofold potentiation. Furthermore, Lebowitz[6] found this combination behaved analogously in humans. It seems reasonable to conclude that one should avoid a heavy hand if using these two agents in the same patient. With the

other combination, gallamine plus metocurine, which we found to show clear potentiation, confirmation that the phenomenon extends to humans is not available. However, it would seem prudent to proceed cautiously here as well. With all the other pairs, there seems little reason to expect enough deviation from simple additivity to be detectable above the noise level of normal patient-to-patient variation.

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