THE MEDIAN PARALYSIS UNIT: A MORE PHARMACOLOGICALLY RELEVANT UNIT OF BIOLOGIC ACTIVITY FOR BOTULINUM TOXIN

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L. B. Pearce, G. E. Borodic, E. A. Johnson, E. R. First and R. D. MacCallum. The median paralysis unit: a more pharmacologically relevant unit of biologic activity for botulinum toxin. Toxicon 33, 217–227, 1995.—Although the LD₅₀ has been used to quantify the biologically active toxin in clinical preparations of botulinum A toxin (Botox® and Dysport®), a discrepancy exists between the clinical potency of equivalent international units of different formulations of botulinum A toxin for multiple clinical indications. Our laboratory previously reported that a regional chemodenervation assay in the mouse could be utilized to detect the difference in the potencies of the clinical preparations of toxin [Pearce et al. (1994) Toxic. appl. Pharmac. 128, 69–77]. The purpose of this study was to quantify the regional paralysis produced by botulinum toxin and define a new pharmacologic/biologic unit of activity that more accurately reflects the mechanism of action of botulinum toxin in the clinical setting. Quantal analysis of regional paralysis revealed that the ED₉₀, defined as the median paralysis unit (MPU) for Botox® and Dysport®, was 0.41 ± 0.01 and 1.00 ± 0.02 LD₅₀ units, respectively. Differences in the potencies found in retrospective clinical studies comparing Botox® and Dysport® were accurately reflected, for the first time, by the dose of toxin expressed in terms of the MPU (median paralysis unit). The data suggested that the MPU may be a more appropriate measure of the biologic activity in therapeutic formulations of botulinum toxin.

INTRODUCTION

Botulinum toxin is a paralytic neurotoxin that has become widely investigated for its therapeutic potential in the treatment of a variety of neuromuscular disorders (Simpson, 1981; Habermann, 1989; Jankovic and Brin, 1991; Borodic et al., 1991; Hambleton, 1992; Schantz and Johnson, 1992; Valtorta and Arslan, 1993). Intramuscular injection of nanogram quantities of purified botulinum toxin is the treatment of choice for a number of clinical indications including: blepharospasm, spasmodic dysphonia, hemifacial spasm, and adult onset spasmodic torticollis. The denervating potency, duration of effect, and
diffusion potential of botulinum toxin are important pharmacologic properties that should be considered when evaluating the biologic activity of clinical preparations of the toxin (Borodic et al., 1990, 1992, 1994). The primary method for the measurement of botulinum toxin activity is estimation of the median lethal dose in mice, the $LD_{50}$, as described by Schantz and Kautter (1977). The international unit of biologic activity of botulinum toxin has been defined as the $LD_{50}$ in mice. This unit of biologic activity provides one measure of the toxic properties of botulinum toxins; however, it does not reflect the pharmacologic properties of botulinum toxins most important in clinical practice.

Comparison of the clinical potency of the two approved preparations of botulinum toxin (Botox®, Dysport®) has raised the question of whether or not the lethality assay is the most accurate method for assessing the biologic activity of botulinum toxin. Recommended doses for the treatment of blepharospasm with Botox® are 10–30 U/eye whereas 100–200 U/eye are recommended for Dysport® (Clarke, 1992). This large disparity in the required dosing is also seen for the treatment of torticollis where total doses of 100–300 U versus 600–1200 U have been reported for Botox® and Dysport®, respectively (Brin and Blitzer, 1993; Clarke, 1992; Zuber et al., 1993). Recently Pearce et al. (1994) examined the factors affecting the precision of the mouse $LD_{50}$ assay for botulinum toxin. The results of these studies indicated that this assay could be used to estimate precisely the lethality, $LD_{50}$, for botulinum toxin. Comparison of the two clinically approved preparations of toxin using the mouse $LD_{50}$ assay revealed that there was almost a two-fold difference in the units of activity defined for Dysport® and Botox®. However, this two-fold difference did not account for the much greater difference in the clinical potency of the two A toxin preparations based on published studies. A regional chemodenervation assay was devised to compare more accurately the clinically important bioactivity parameters and further evaluate the relative potencies of the two preparations of toxin. Once normalized for the discrepancy in the $LD_{50}$ units of activity of the British and American toxins, an additional 2.4-fold difference in the two preparations was observed. Hence, the reported difference in the clinical potency of the two preparations of botulinum A toxin that was not detectable by the standard $LD_{50}$ assay was revealed by a regional chemodenervation assay (Pearce et al., 1994).

The results presented below demonstrate that a regional chemodenervation assay better approximated the clinical use of the toxin. We propose a new unit of pharmacologic/biologic activity for botulinum toxin, the median paralysis unit, which accurately predicts the differences in clinical potency of the British and American botulinum A toxins approved for clinical use.

**MATERIALS AND METHODS**

CD1 white male mice, weighing 18–22 g, and free from viruses and antibodies, were obtained from Charles River Laboratories. Type A porcine skin gelatin was obtained from Sigma Chemical Co. (St Louis, MO, U.S.A.). Botox® (Oculinum®) was obtained from Allergan Pharmaceuticals (Irvine, CA, U.S.A.) and Dysport® was obtained from Porton Down Ltd (U.K.).

*The hind limb regional chemodenervation assay*

Paralysis of the mouse hind limb was produced by i.m. injection of botulinum toxin in a manner similar to that described by Sugiyama et al. (1975). Toxin was diluted with 0.2% gelatin in 30 mM sodium phosphate buffer, pH 6.2, and 0.1 ml of diluted toxin in preparations was injected into the gastrocnemius muscle of the hind limb of 18–22 g mice. The amount of toxin activity in a given preparation was assessed by evaluating the fraction of mice that showed complete paralysis of the right hind limb. Complete paralysis was defined as the inability of the mouse to use the right rear hind limb to support his weight or to escape. Once complete paralysis occurred
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the hind limb was usually held up against the body or dragged; these postures were considered cardinal signs of what was defined as complete paralysis.

Five to six dilutions of toxin were injected into ten mice per dilution. Doses of toxin were increased in a geometric progression, by a factor of 1.25. Attempts were made to center the doses on the ED₉₀ to provide a symmetric design (Finney, 1978). To reduce bias, only doses of toxin at which no deaths occurred prior to determining the ED₉₀ were utilized in the probit analysis. The percentage paralyzed was determined at each dose of toxin and probit analysis was performed on the data using the probit (Bliss, 1938) program provided with the statistical package SPSS-X (SPSS, Inc., Chicago, IL, U.S.A.). This program estimates the best line by regression analysis and the values for the intercept and slope are estimated by the maximum likelihood method. The 95% fiducial confidence intervals for the estimates of the LD₉₀ (Trevan, 1927) were determined as described by Finney (1971). A Pearson Chi-square goodness of fit test was used, and if this estimate was significant, a heterogeneity factor was used in the calculation of the confidence limits.

RESULTS

Probit analysis of regional chemodenervation produced by botulinum toxin

The applicability of the paralysis assay for the measurement of the denervating activity of botulinum toxin was investigated. Following injection of picogram quantities of botulinum toxin into the gastrocnemius muscle (calf muscle), the fraction of the mice exhibiting complete paralysis of the injected hind limb was recorded. The dose of toxin was expressed in terms of the number of LD₉₀ units. The data obtained from experiments in which the dose dependence of complete paralysis was investigated are shown in Fig. 1. In panel A the percentages of mice exhibiting complete paralysis at five different doses of either Botox® and Dysport® are shown. The corresponding probit analysis of these same data are presented in panel B. The results of these experiments show that the ED₉₀ ± S.D., expressed in terms of LD₉₀, was 0.41 ± 0.01 for Botox® and 1.00 ± 0.02 for Dysport®. In the text below the ED₉₀ obtained from this type of experiment is specifically referred to as the median paralysis unit (MPU). Comparison of the potency ratio based on the MPU values for Dysport® and Botox® revealed a 2.44-fold difference in potency between these two formulations of A toxin.

**Fig. 1.** Analysis of the dose dependence of the hind limb paralysis produced by botulinum A toxins. White CD1 18–22 g male mice received 0.1 ml i.m. injections in the gastrocnemius (calf) muscle of the right hind limb of two preparations of botulinum A toxin, Dysport® (●●●) and Botox® (○○○). Animals were monitored for 6 days and the percentage of mice demonstrating complete paralysis of the injected hind limb was evaluated on day 2 for Dysport® and 3 for Botox®, as shown in panel A. The results of probit analysis performed on the same data are shown in panel B. Error bars represent the S.E.M. obtained from three experiments in which ten mice were studied at each dose.
Table 1. The relationship between number of doses and the estimated MPU

<table>
<thead>
<tr>
<th>Preparation of toxin</th>
<th>Number* of doses</th>
<th>MPU† ± S.D.‡ (LD₉₀ units)</th>
<th>C.V. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Botox*</td>
<td>6</td>
<td>0.414 ± 0.007</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.425 ± 0.008</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.421 ± 0.003</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.38 ± 0.01</td>
<td>3</td>
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<td></td>
<td>3</td>
<td>0.38 ± 0.01</td>
<td>3</td>
</tr>
</tbody>
</table>

* Ten animals at each dose.
† The MPU was expressed in terms of the number of LD₉₀ units of toxin. The LD₉₀ units were defined by lethality bioassays performed on these samples.
‡ S.D. was based on the results of three bioassays.

Precision of the regional chemodenervation assay

Table 1 contains a summary of the relationship between the number of doses of toxin used in the estimation of the MPU and the precision of multiple determinations. These data show that reduction of the number of doses from six to three results in an increase of the coefficient of variation from 2% to 4%. Although this represents a doubling of the standard deviation, the results obtained with three doses of toxin still provided a precise estimate of the MPU.

Duration of complete paralysis

It is well known that the chemodenervating effects of botulinum toxin are long lived but reversible over time. Accordingly, following i.m. administration of picograms of toxin mice

![Graph showing the time course of paralysis produced by Botox*](image)

Fig. 2. The time course of paralysis produced by Botox*. White CD1 18–22 g male mice were administered 0.24 (□—□), 0.30 (▼—▼), 0.38 (○—○), 0.47 (■—■), 0.59 (◇—◇), or 0.74 (●—●) mouse LD₉₀ units of Botox* by i.m. injection into the gastrocnemius muscle of the right rear hind limb. The percentage of the mice showing complete paralysis following injection is presented as a function of time. Ten mice were used at each dose and error bars represent the S.E.M. from three experiments.
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Fig. 3. Time course of paralysis produced by Dysport®.
White CD1 18–22 g male mice were administered 0.59 (▽—▽), 0.74 (○—○), 0.92 (■—■), or 1.15 (▼—▼) mouse LD₅₀ units of Dysport® by i.m. injection into the gastrocnemius muscle of the right hind limb. The percentage of the mice showing complete paralysis following injection is presented as a function of time. Ten mice were used at each dose and error bars represent the S.E.M. from three experiments.

were monitored until complete paralysis resolved, and the time course of this effect was examined for several doses of toxin. The results of experiments in which the duration of complete paralysis was studied as a function of dose for both Dysport® and Botox® are shown in Figs 2 and 3. These results demonstrate that both the maximum effect and the duration of the effect were a function of the dose of toxin expressed in MPUs. The relationship between dose and duration was examined further, as shown in Fig. 4 where the log of the dose in LD₅₀'s and MPUs was plotted against the duration of action in panels A and B, respectively. This figure illustrates the transformation of the relationship between the toxins when the data are converted from LD₅₀ units to MPUs. Several parameters were

Fig. 4. The duration of effect as a function of the log dose.
The maximum duration of complete paralysis was determined from the data plotted in Figs 2 and 3. The maximum duration is plotted as a function of the dose expressed in terms of LD₅₀'s in panel A and MPUs in panel B. Plots for both Dysport® (■—■) and Botox® (○—○) are presented in each panel. The error bars represent the S.E.M. obtained from at least three experiments in which ten mice were studied at each dose.
Fig. 5. The effect of time on estimation of the MPU.
White CD1 18–22 g male mice received 0.1 ml i.m. injections in the gastrocnemius muscle of the right hind limb of two preparations of botulinum A toxin, Dysport® and Botox®. Animals were monitored for 5 days and the percentage of mice demonstrating complete paralysis of the injected hind limb was evaluated. The results of probit analysis performed on days 1–6 or 7 are plotted for Botox® (O—O) and Dysport® (●—●), respectively. The results are expressed as the number of LD₉₀ units required to produce an effect equivalent to 1.0 MPU. Error bars represent the S.E.M. of the data obtained from three experiments.

obtained from the analysis of the data plotted in this manner. The plots for the toxins intersect the y axis at the point at which the log dose is zero, that is, at 1.0 LD₉₀ or 1.0 MPU. Thus, the duration of action was determined at a standardized dose of botulinum A toxin. The duration of action at 1.0 LD₉₀ for Botox® was 7.9 ± 0.2 days, whereas it was 6.4 ± 0.5 for Dysport®. The difference in these values was statistically significant at P = 0.015. In contrast, the duration of action for Botox® at 1.0 MPU was 5.0 ± 0.1 days and 5.1 ± 0.3 days for Dysport®. These intercepts were not significantly different (P = 0.91). The slopes for the regression lines for Botox® and Dysport® were significantly different for the plots shown in panels A and B.

Analysis of the MPU as a function of duration of the assay
The data presented in Fig. 5 show that the MPU value was a function of time. This is consistent with the observation that complete paralysis produced by localized injection of botulinum toxin was a transient effect, as shown in Figs 2 and 3. The data indicate that the minimum MPU value occurred on day 2 for Dysport® and day 3 for Botox®, which correspond to the days on which the maximal paralytic effect occurred. The comparisons between the two formulations of toxin shown in Fig. 1 were based on the minimum MPU values. These data indicate the importance of the transient nature of the duration of action of botulinum toxin on accurate calculation of the MPU required to permit valid comparisons of potency.

DISCUSSION
A number of methods have been employed to measure the biologic activity associated with botulinum neurotoxins (Schantz and Kautter, 1977; Notermans and Nagel, 1989;
Mayorga et al., 1987; Sugiyama et al., 1975). The median lethal dose, $LD_{50}$, has been used to define the basic biologic unit of activity of botulinum toxin and has assumed the distinction of being the gold standard. However, there are limitations associated with using the $LD_{50}$ as a measure of the biologic activity relevant to the clinical use of botulinum neurotoxins. This is best illustrated by the marked discrepancies in the number of $LD_{50}$ units of toxin required to achieve an equivalent therapeutic effect of Botox® and Dysport® (Brin and Blitzer, 1993; Marsden, 1993). From a clinical perspective, the desired effect of treatment with botulinum toxin is regional chemodenervation within a muscle or muscle group. Accordingly, we investigated an assay that utilized regional chemodenervation to assess the biologic activity of botulinum toxin in a manner that better approximated the pharmacologic effect observed in clinical practice.

Pearce et al. (1994) reported that injection of very low doses of Botox® and Dysport® into the gastrocnemius muscle of the mouse hind limb and assessment of the degree of paralysis made it possible to demonstrate differences in the potencies of the two A toxin preparations licensed for clinical use. In this previous study paralysis was analyzed as a continuous variate response by scoring the degree of paralysis, whereas in the results presented herein paralysis was treated as a quantal response and the percentage of the population of mice demonstrating complete paralysis of the injected hind limb was tabulated. Measurement of muscle paralysis to evaluate botulinum toxin activity has been employed by a number of investigators (Duchen and Strick, 1969; Sugiyama et al., 1975; Sellin et al., 1983). However, the quantitative differences between regional paralysis and the $LD_{50}$ have never been established. Our approach was based, in large part, on that described originally by Sugiyama et al. (1975), who developed a local paralysis assay to measure botulinum toxin in different foods. The signs of local paralysis described by Sugiyama et al. (1975) were the same as those observed in our laboratory following toxin injection into the gastrocnemius muscle. Unlike those authors, however, complete paralysis of the right hind limb was used as the endpoint for the assay. There were several reasons for using this particular endpoint. First, complete paralysis is an unambiguous effect and one that can be easily assessed. Second, it was hoped that this clearly definable endpoint would improve the precision of the assay. The low coefficient of variation for estimation of the MPU, as low as 1–2%, was evidence of the high degree of reproducibility possible using this endpoint.

The precision of estimates of the MPU using the mouse hind limb paralysis assay was better than that observed in our laboratory for estimates of the $LD_{50}$ in mice (Pearce et al., 1994). The coefficient of variation obtained using the same numbers of doses or animals is two-fold lower for the estimation of the MPU. Although experiments were not performed using less than ten animals per dose, the observed data suggest that statistically satisfactory estimates of biologic activity could be obtained with fewer than ten animals per dose. Thus, the number of animals used in this report should not be adopted without examining the possibility of further reducing the number of mice used at each dose. The use of animals in basic research and in particular the testing of commercial products has come under considerable criticism. Clearly, the required precision of the measurement of biologic activity should determine the number of mice employed for a given assay. Very few animals would be required for a rough estimate of the MPUs in a given preparation of toxin utilized in the laboratory, whereas a relatively high degree of precision would be required for characterization of clinical preparations of botulinum toxin. In addition to reducing the number of animals needed, this approach reduces the suffering that is an unavoidable consequence of the $LD_{50}$ analysis. The interlaboratory variability of bioassays
can be substantial and the mouse hind limb assay will require the same standardization as any other bioassay. Thus, when comparisons between laboratories are undertaken efforts should be made to include a reference standard, as proposed by Schantz and Kautter (1977) for the mouse lethality assay.

Interest in the biologic activity of botulinum toxins has evolved from an initial emphasis on the toxic properties of the neurotoxins associated with accidental poisoning to the current focus on the therapeutic applications. The mouse lethality assay was and remains the most appropriate method for predicting the biological consequences of exposure to extremely high doses of toxin, as commonly occurs in food poisoning. In sharp contrast, interest is currently focused on the pharmacology of point injection of extremely low doses, nanograms of botulinum toxins. Treatment with such small amounts of toxin results in partial denervation in humans (Borodic et al., 1993). It follows that an assay which measures the denervating effects of low doses of toxin would be more relevant to the therapeutic properties of the toxin. One of the most significant observations of the studies described above was that the difference in the clinical potencies of Botox® and Dysport® correlated with the difference in potencies of these two formulations as defined by the MPU. Clearly, the data demonstrating that the mouse hind limb assay distinguishes the difference in the potencies of the two toxins suggest that this bioassay provides a superior method for the definition of the clinically relevant biologic activity of botulinum toxin.

Brin and Blitzer (1993) have recently pointed out that the confusion regarding the recommended doses for the two approved preparations of botulinum A toxin could have serious consequences, particularly if authors are not careful to distinguish clearly between these formulations. The lack of correlation between the recommended doses (IUs) of Botox® and Dysport® has led to considerable confusion over the concept of potency, as it applies to botulinum toxin. The term potency is used to refer to the relative amounts of two drugs required to produce an equivalent effect, and the convention is to express the amounts of drug in terms of mass, milligrams, etc. On the basis of this convention it would be appropriate to express the dose of botulinum toxins on the basis of the number nanograms of toxin that produce a standard (equivalent) therapeutic effect. However, the amount of activity per nanogram (specific activity) can vary from preparation to preparation of toxin. Thus, expressing the dose of toxin without knowledge of the specific activity of the toxin may be meaningless, as pointed out by Schantz and Johnson (1990). The approach advocated by Schantz and Johnson (1990) to obviate this problem was to express the dose of biologic units of toxin activity, that is, in terms of LD<sub>50</sub> units defined by a standardized mouse lethality assay (Schantz and Kautter, 1977). Pearce et al. (1994) have recently provided additional evidence of the importance of standardizing the LD<sub>50</sub> assay used to define the number of units of biologic activity in a given preparation of toxin. A 1.92-fold difference in the units of activity defined for the British and American toxins was demonstrated. It is important to point out that while these preparations of toxin are labelled as containing a particular number of units of toxin, there is a certain allowable error with regard to the actual content of toxin of up to 30% in the case of Botox®. Accordingly, some of this difference in the apparent units defined for the British and American toxins might be accounted for on the basis of the fact that these vials may not contain exactly what the label indicates.

Standardization of the LD<sub>50</sub> assay can account for some of the difference in the clinical potency of the two toxins; however, this is only one of the reasons for this disparity. The results presented herein clearly indicate that the relative potency of different preparations of botulinum toxin cannot be accurately estimated by comparison of the LD<sub>50</sub> values. To
clarify further the relationship between the \(LD_{50}\) and MPU as fundamental units of the biologic activity of botulinum toxin, the relationship between the British and American toxins is examined in Table 2. The \(LD_{50}\) units in each preparation of toxin shown in Table 2 were corrected values obtained from estimations of the \(LD_{50}\) units performed in this laboratory. Thus, the number of 'observed' \(LD_{50}\) units has been adjusted for the 1.92-fold difference in the definition of the units of activity. On the basis of these estimates of the number of \(LD_{50}\) units in each vial of toxin, the number of MPUs per vial was estimated for Dysport® and Botox®. The most striking result of this comparison was that there were approximately equal total MPUs in the two preparations of toxin, and not the five-fold difference determined on the basis of the \(LD_{50}\) units. That is, on the basis of MPUs there was approximately the same amount of denervating activity in the two formulations. It is important to realize that two factors have contributed to the aggregate 4.7-fold difference in the potencies of Dysport® and Botox® reported herein. First, there is a 1.92-fold difference in the definition of the \(LD_{50}\) units (Pearce et al., 1994) and second, there is a 2.44-fold difference in potency determined on the basis of the MPU values.

The duration of action is one of the fundamentally most important characteristics of botulinum toxin because repeated injections are necessary to treat chronic disease and frequent toxin exposures are related to immunologic resistance (Scott, 1990; Zuber et al., 1993; Greene and Fahn, 1993). This is particularly important because of the unusually long duration of action (10–14 weeks) following the i.m. administration. It is becoming apparent that the different serotypes of the toxin may have distinctly different durations of action. Greene and Fahn (1993) recently reported that the duration of the therapeutic effect following F toxin injections lasted only about 1 month, compared to 3 months observed for A toxin. Thus, in addition to characterization of preparations of the toxin in terms of the chemodenervating potency, it would be advantageous to have a parameter that characterizes the duration of action of a given toxin. With this issue in mind the relationship between duration of action and the dose of toxin was explored. If the duration of action is plotted as a function of dose expressed in terms of log \(LD_{50}\) or log MPU, the \(y\) intercept value corresponds to the duration of action at 1.0 \(LD_{50}\) (panel A) or 1.0 MPU (panel B). Thus, the duration of action can be defined at a standardized level of effect. The data obtained from this analysis revealed that when the log \(LD_{50}\) data were used the duration of action of Botox® and Dysport® was statistically significantly different. However, the duration of effect was identical for Botox® and Dysport® when evaluated on the basis of 1.0 MPU.

The current thinking with regard to the mechanism responsible for recovery from chemodenervation by botulinum toxin suggests that this is dependent upon the sprouting of neurons and reestablishment of neuromuscular junctions (Duchen and Tonge, 1977; Aldersen et al., 1989; Holds et al., 1990; Alderson et al., 1991; Borodic et al., 1994).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Labelled (LD_{50}) units per vial</th>
<th>Observed* (LD_{50}) units per vial</th>
<th>(LD_{50}) MPU per vial</th>
<th>MPUs per vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Botox®</td>
<td>100</td>
<td>123</td>
<td>0.414</td>
<td>297</td>
</tr>
<tr>
<td>Dysport®</td>
<td>500</td>
<td>330</td>
<td>0.998</td>
<td>331</td>
</tr>
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</table>

* The number of actual units in each vial determined by the mouse \(LD_{50}\) assay.
If a single process like this is responsible for the recovery from denervation then it would be expected that the level of effect, the extent of denervation, should dictate the duration of the effect. Furthermore, this relationship should be the same for all types of botulinum toxin. The results of clinical studies with the F toxin reported by Greene and Fahn (1993) would suggest otherwise. It is difficult to rationalize the observed differences in the duration of action of the A and F toxins on the basis of a single mechanism such as resprouting that results in the formation of new neuromuscular junctions. However, the relationship between the MPUs and LD50's for botulinum F toxin is not known and it would be interesting to compare the duration of mouse hind limb paralysis observed at 1.0 MPU of F toxin to that reported here for botulinum A toxin. Preliminary results in our laboratory suggest that the duration of action of botulinum B toxin at 1.0 MPU was not different from the A toxins examined to date. The data with the A and B serotypes suggest that the MPU defines a unit of activity that also defines a particular duration of action. The statistically significant differences in the slopes of the log MPU versus duration of action plots shown in Fig. 4 suggest that perhaps the same durations of action observed at 1.0 MPU might be purely coincidental. Whether the unit of biologic activity defined by the MPU also defines a specific duration of action will need to be tested further by examination of a variety of serotypes and strains of botulinum toxins.

Regional chemodenervation is apparently responsible for the therapeutic effect observed following i.m. administration of botulinum toxin. Consistent with this proposed mechanism, the results of this study suggest that the therapeutic potency of botulinum toxins is more accurately predicted by directly measuring regional denervation rather than lethality in laboratory animals. The MPU is a standardized dose of toxin that produces a specific degree of regional denervation measured in terms of the paralysis observed following i.m. administration to the mouse hind limb. Accordingly, the MPU should be examined for its potential as a standardized unit which defines the denervating activity in clinical preparations of botulinum toxin. At the very least, definition of the clinical potency of botulinum toxin formulations in terms of the MPU or some other standardized measure of localized chemodenervation will help to rectify the current disparity in the clinical potencies of Botox® and Dysport®.

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REFERENCES


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