Supplemental Figures.

Supplemental Figure 1. **Dose-dependent toxicities following single and multiple administration of 3,4-DAP to naïve mice.** Mice (n = 5 per dose) were administered single doses of 0.5-16 mg/kg 3,4-DAP and observed for behavioral markers of adverse effects over 4 h. To characterize putative central nervous effects, median behavioral signs of seizure were estimated using the Racine scale (81). The percentage of mice exhibiting signs consistent with Racine score ≥ 1 and the median score among the responders within each dose group are reported. The multiple-dosing treatment was 2 mg/kg, administered fifteen times at 90 min intervals.

Supplemental Figure 2. **Pharmacokinetic (PK) modeling of 3,4-DAP plasma levels following single subcutaneous administration of 2 mg/kg 3,4-DAP.** Mice (n = 8 per time point) were administered 2 mg/kg 3,4-DAP and serum was collected for analysis at 0, 5, 10, 20, 40, 60, 120, 240 and 480 min. (A) 3,4-DAP plasma levels were quantified via liquid chromatography – mass spectrometry, and pharmacokinetic parameters were calculated using a two-compartment PK model. (B) Input-output comparison demonstrating linearity and sensitivity of analytical method used for PK analyses. (C) Tabular summary of PK parameters. (D) Predicted 3,4-DAP plasma levels during 15 consecutive administration of 2 mg/kg 3,4-DAP given at 1.5 h intervals. The dashed line at 50 ng/mL corresponds to the estimated minimum therapeutic concentration. These estimates assume the absence of a saturation effect at these doses and plasma concentrations, consistent with clinical pharmacokinetic studies (47).

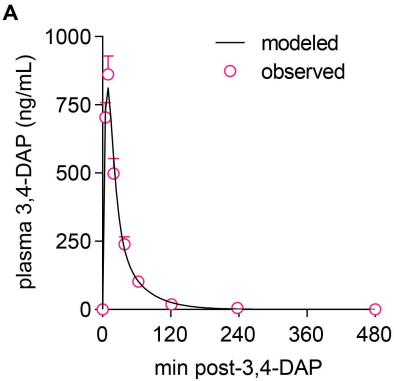
Supplemental figure 3. Toxicokinetic and pharmacodynamic evaluation in mice treated with 3,4-DAP and BoNT serotypes A, B and E. Mice maintained in metabolic cages were treated with 1.5 LD_{50} BoNT/A (n = 8), 1.5 LD_{50} BoNT/B (n = 8), 1.25 LD_{50} BoNT/E (n = 8), 1.5 LD_{50} BoNT/A co-administered with 10,000-fold molar excess of sheep anti-BoNT/A antitoxin (/A + IgG; n = 8), or 3,4-DAP (2 mg/kg) and monitored over time. **(A-C)** Longitudinal changes in CSS **(A)**, VO2 **(B)** and Kaplan-Meier survival curves **(C)** in intoxicated mice as a function of time after intoxication. Onset of respiratory botulism was defined to be when VO_2 declined more than three standard deviations below experimentally determined resting VO_2 (annotated by horizontal dashed line in panel B; see methods). The absence of toxic effects in antitoxin-treated mice confirmed that respiratory depression was specific to BoNT intoxication. **(D)** Tabular comparison of the start of VO_2 decline, emergence of respiratory signs and TD_{50} (determined from Kaplan-Meier curves) among serotypes A, B and E. PAB = paradoxical abdominal breathing; AR = agonal respiration. (**E,F**) 3,4-DAP (2 mg/kg) transiently increased mean VO_2 in naïve mice within 34 min, whereas vehicle treatments had no effect (n = 8, two-way repeated measures ANOVA with Sidak's multiple comparisons test, $p < 0.0001$).

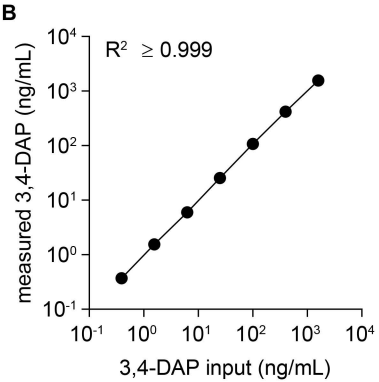
Reference

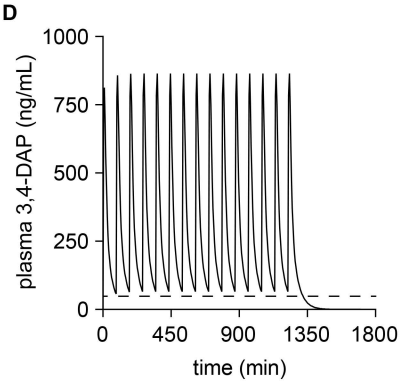
81. Lüttjohann A, Fabene PF, van Luijtelaar G. A revised Racine's scale for PTZ-induced seizures in rats. *Physiol Behav*. 2009;98(5):579–586.

Single and multiple dose-dependent 3,4-DAP toxicity in mice (n = 5 per condition)

3,4-DAP (mg/kg)	gait change (%)	salivation (%)	behavioral seizure (%), [median Racine score]	mortality (%)
0	0	0	0	0
0.5	0	0	0	0
2	0	0	0	0
4	0	0	0	0
8	40	80	60 [I]	0
16	100	100	100 [II]	0
2 (x15)	0	0	0	0





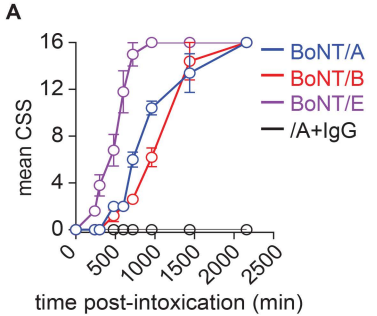


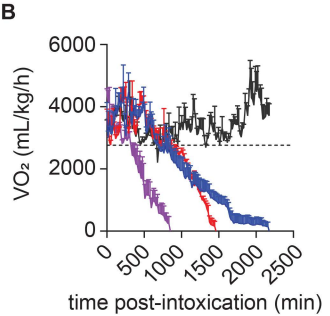
C

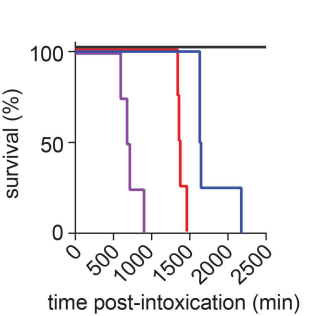
Two-compartment pharmacokinetic modeling (avg mouse weight 28.6 ± 2.2 g)

pharmacokinetic parameter	value
absorption phase half-life (min)	2.8
distribution phase half-life (min)	5.3
terminal phase half-life (min)	29.4
V/F central compartment (mL/kg)	1034.7
CL/F central compartment (mL/min/kg)	72.4
V2/F peripheral compartment (mL/kg)	949.1
CL2/F peripheral compartment (mL/min/kg)	42.0
Tmax (min)	8.4
Cmax (ng/mL)	820.7
AUC 0-inf (ng·h/mL)	460.3
MRT (min)	34.5

diagnostic parameter	value
r obs-pre (%)	99.6
SS	6758.2
R^2 (%)	99.6
SE	41.1
AIC	89.4
SC	90.4







D

	symptomatic emergence			
challenge	VO_2 (min)	PAB (min)	AR (min)	TD_{50} (min)
1.5 U /A	714	480	900	1649
1.5 U /B	799	420	960	1377
1.25 U /E	323	240	420	697
1.5 U /A+IgG	*nd*	*nd*	*nd*	*nd*

