Supplementary Data

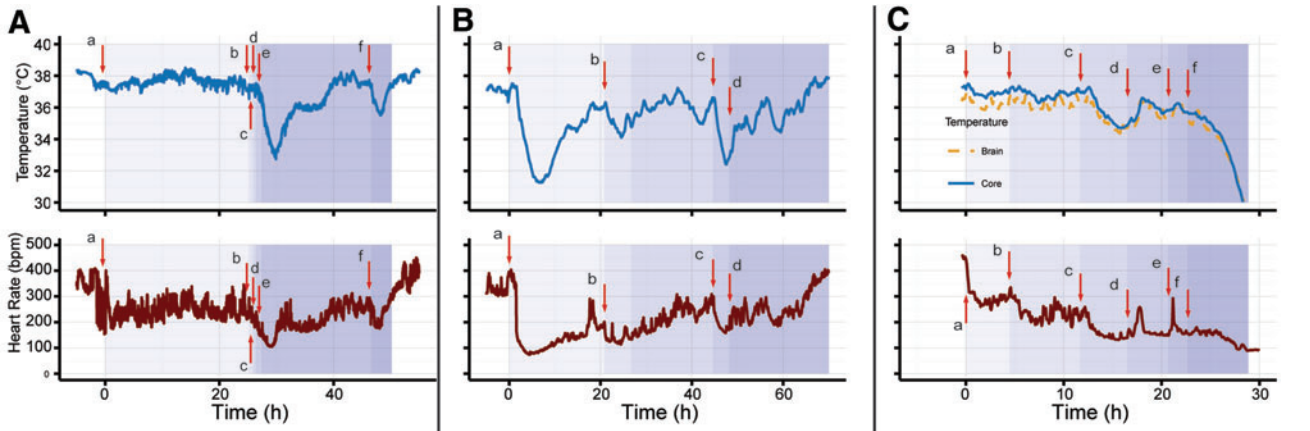
Supplementary Figure S1A–C depicts results from three representative experiments that illustrate development of tolerance and failure for dose titration to produce the desired effect.

We show that doubling the IV dose of N^6-cyclohexyladenosine (CHA) four consecutive times had no effect on core temperature (Supplementary Fig. S1 A: arrows: a–d). A subsequent IP injection of 0.5 mg/kg (arrow: e) produced a large drop in core T_b that was not sustained despite continuous IV infusion of 0.4 mg/(kg·h). One hour later 0.5 mg/kg CHA delivered via IV bolus (arrow: f) resulted in a smaller drop in core T_b than the preceding IP bolus.

Similarly, in another experiment (Supplementary Fig. S1B), we observed tolerance with two IP loading doses separated by 20 hours. A loading dose of 0.5 mg/kg, IP, followed by continuous IV infusion (arrow: a) resulted in a large and sustained drop in T_b. Repeating the same IP loading dose and doubling the dose of continuous IV infusion (arrow: b) produced a smaller fall in T_b than in (arrow: a). Increasing the dose of CHA delivered via continuous IV infusion fivefold, 20 hours later, produced a 4°C drop in T_b, but the lower T_b was not sustained despite continuous infusion (arrow: c). A final IP injection, twice the original dose (arrow: d), failed to decrease T_b.

Finally, (Supplementary Fig. S1C) illustrates an example where increasing the dose of IV infusion at intervals of less than 12 hours eventually produces a significant decrease in T_b, but in this case T_b decreased below the target T_b of 30–32°C. In this animal, both T_{brain} and core T_b were measured. An initial dose of 0.025 mg/(kg·h) IV CHA at an ambient temperature of 17°C had little effect on T_{brain} or core T_b during 4.4 hours of infusion. Doubling the continuous IV dose failed to increase the response to CHA after 7.3 hours. When the dose was doubled, a second time over 4.8 hours T_{brain} and core T_b decreased slightly. Further increase in dose had no additional effect on brain or core T_b over 4.2 hours of continuous infusion. Further doubling the dose again failed to decrease T_{brain} or T_b over the next 2 hours. The final doubling of dose produced a 6°C decrease in T_{brain} and core T_b over 6 hours.

In all six experiments in which T_{brain} and T_b were monitored in the same animal, T_{brain} decreased at the same rate as T_b, as shown in this representative example. These results show that titrating the dose of CHA fails to produce a consistent or controlled decrease in T_b.



CHA Dose mg/kg/h IV or mg/kg IP	Infusion duration (h)	T_{core} (°C)	HR (bpm)	$T_{ambient}$ (°C)
Baseline	NA	37.9 ± 0.004	367.0 ±0.46	24.1 ± 0.02
[a] 0.025 IV	25.17	37.7 ± 0.003	241.8 ± 0.31	23.9 ± 0.02
[b] 0.05 IV	0.75	37.3 ± 0.006	232.8 ± 0.91	24.5 ± 0.13
[c] 0.25 IV	0.50	37.3 ± 0.007	205.8 ± 0.86	24.3 ± 0.17
[d] 0.5 IV	1.00	37.0 ± 0.014	179.7 ± 1.33	24.6 ± 0.00
[e] 0.5 IP + 0.4 IV	19.25	36.3 ± 0.017	209.5 ± 0.61	23.5 ± 0.11
[f] 0.5 IV bolus + 0.125 IV continuous	3.50	36.4 ± 0.017	231.5 ± 1.01	21.4 ± 0.12

CHA Dose mg/kg/h IV or mg/kg IP	Infusion duration (h)	T_{core} (°C)	HR (bpm)	$T_{ambient}$ (°C)
Baseline	NA	37.5 ± 0.031	358.1 ± 3.04	20.3 ± 0.05
[a] 0.5 IP + 0.05 IV	20.9	34.3 ± 0.115	151.2 ± 4.76	19.5 ± 0.03
[b] 0.5 IP + 0.10 IV	5.9	35.0 ± 0.678	137.9 ± 2.19	19.9 ± 0.04
[c] 0.5 IV	17.9	36.0 ± 0.381	219.2 ± 3.02	19.8 ± 0.02
[d] 1.0 IP + 0.125 IV	3.57	34.0 ± 0.211	189 .1 ± 4.61	20.1 ± 0.00

CHA Dose mg/kg/h IV or mg/kg IP	Infusion duration (h)	T_{core} (°C)	T_{brain} (°C)	HR (bpm)
Baseline	NA	37.3 ± 0.04	36.7 ± 0.04	366.9 ± 17.4
[a] 0.025 IV	7.25	36.9 ± 0.02	36.2 ± 0.02	286.7 ± 2.4
[b] 0.05 IV	4.83	36.8 ± 0.03	36.3 ± 0.02	230.1 ± 2.7
[c] 0.1 IV	4.17	35.5 ± 0.10	35.2 ± 0.04	151.4 ± 2.2
[d] 0.2 IV	2.00	36.01 ± 0.05	35.7 ± 0.03	175.6 ± 5.0
[e] 0.4 IV	6.00	35.8 ± 0.05	35.5 ± 0.04	161.4 ± 1.7
[f] 0.8 IV	1.30	32.9 ± 0.27	32.7 ± 0.11	132.8 ± 3.4

SUPPLEMENTARY FIG. S1. Three representative samples depicting the development of tolerance under various CHA administration protocols. Start of CHA administration is depicted by *arrows*, and change in dose is correlated with change in *background color* and *letters* (a–f) in the table. Table columns correspond to graphs immediately above each *column*. Values in table are mean ± SEM for the duration of dose. **(A)** Protocol of increasing the IV dose six times and administration of both and IP and IV bolus. **(B)** Protocol of IP loading dose followed by continuous IV dose. **(C)** Protocol of sequentially doubling the IV dose. Both brain and core body temperature decrease at the same rate. Representative example of T_{brain} and T_b monitored in the same animal shows that T_{brain} decreases at the same rate as T_b. CHA, N^6-cyclohexyladenosine; T_b, body temperature; T_{brain}, brain temperature.