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Resumo	*Bothrops fonsecai* (*B. fonsecai*), a pitviper endemic to southeastern Brazil, has a venom mainly composed by snake venom phospholipases (PLA_2) and metalloproteases, compounds that could interfere with neuromuscular junction *in vitro*. In this work, we investigated the role of PLA_2 in the myotoxicity and neuromuscular blockade caused by *B. fonsecai* venom using different procedures frequently associated with PLA_2 activity inhibition: 24 °C bath temperature, Ca^{2+} - Sr^{2+} replacement and chemical modification with *p*-bromophenacyl bromide (*p*-BPB). Mice *extensor digitorum longus* preparations (EDL) were incubated with usual or modified Tyrode solution (prepared with Ca^{2+} or Sr^{2+} respectively) at 24 °C or 37 °C (as controls) and in addition of *B. fonsecai* venom (100 µg/mL) alone or after its incubation with buffer (24 h, 23 °C) on the absence (alkylation control) and presence of *p*-BPB; all muscle were processed for histological analysis. The PLA_2, proteolytic and amidolytic activities under the same conditions (24 °C or 37 °C, Ca^{2+} - Sr^{2+} replacement, absence or presence *p*-BPB) were also assessed. The *B. fonsecai* venom caused total neuromuscular blockade after 100 min of incubation, in Ca^{2+} Tyrode solution at 37 °C (usual conditions); on Sr^{2+} Tyrode solution (37 °C) the twitch height were 31.7 ± 7.4% of basal, and at 24 °C (Ca^{2+} Tyrode solution) were 53.6 ± 7.0% of basal. The alkylation of PLA_2 with *p*-BPB promoted a great blockade decrease at 100 min of incubation (88.7 ± 5.7% of basal), but it was also observed on alkylation control preparations (66.2 ± 6.6%). The venom produced 50% of blockade at 40.5 ± 5.9 min, in Ca^{2+} Tyrode solution at 37 °C. The protocols delayed the time for 50% blockade: 105.7 ± 7.1 min (at 24 °C, in Ca^{2+} Tyrode solution) and 71.1 ± 9.0 min (at 37 °C,

	in Sr^{2+} Tyrode solution). Regarding *p*-BPB incubation and alkylation control preparations, 50% of blockade was not reached during the 120 min of venom incubation. Regarding to enzymatic activities, the 24 °C protocol reduced not only PLA_2 (to 62.3%) but also proteolytic (52.3%) and amidolytic (73.4%) activities, as well as observed on *p*-BPB alkylation protocol which markedly inhibited all enzymes (<10%). The alkylation control promoted the same proteolytic and amidolytic inhibition but no reduction of PLA_2 activity; Ca^{2+} - Sr^{2+} replacement reduced only the PLA_2 activity (to 15.3%). We observed a strict relation between the inhibition of PLA_2 activity and the myotoxicity. On the other hand, this relation was not observed with neuromuscular blockade, suggesting that blockade and muscle damage may not be strictly related. It suggests that the neuromuscular blockade may be induced by non-catalytic PLA_2 or other venom components, such as metalloproteinases.
Fomento	