Hypaconitine, the Dominant Constituent Responsible for the Neuromuscular Blocking Action of the Japanese-Sino Medicine "Bushi" (Aconite Root)

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Abstract—The neuromuscular blocking actions of several constituents extracted from Japanese-sino medicine, aconite, were compared in mouse phrenic nerve-diaphragm muscle preparations. Hypaconitine (HAT) was more potent than aconitine (ATN), mesaconitine (MAT) and deoxyaconitine. Lipohypaconitine, coryneine and lipodeoxyaconitine were less effective. Lipoaconitine, benzoylmesaconine, higenamine, kobusine and chasmanine were not effective. The blockades by HAT, ATN and MAT were not recovered by neostigmine. The mechanisms of blockade were similar to that of aconite crude extract. These results suggest that aconite action is dependent on HAT, a main constituent.

The Japanese-sino medicine "bushi" (aconite root) has been frequently used in Kanpō-Hōzai (traditional Japanese-sino prescription) for the purpose of relieving muscular pain. One of the Kanpō-Hōzai, Keishi-ka-zyutubu-tō, was previously reported to depend on aconite which plays an important role in the relaxing action of skeletal muscle (1). Aconite contains many constituents classified chemically as shown in Fig. 1. The aconitine (ATN)-type alkaloids, especially ATN, have shown a wide range of pharmacological actions (2, 3). ATN is highly toxic (4). The chasmanine-, delcosine- and atisine-type alkaloids are constituents with low toxicity, but their pharmacological actions have not been elucidated (5). Coryneine (CRN) (6) and higenamine (HGA) (7, 8) are considered as the main constituents of aconite inducing the hypertensive action and the cardiotonic one, respectively. The neuromuscular blocking effect of aconite is considered to be caused mainly by ATN, but no studies have been done on the effects of the other constituents.

The toxicity in raw aconite root has been reduced by a process of various treatments so that it can be safely used for therapy. The processing converts the ATN-type alkaloids into the much less poisonous benzoylaconines (2) and lipo-alkaloids (9–11), of which the pharmacological activities are considered to be weak (2, 3, 10). The present studies were performed to obtain evidence on the other pharmacologically dominant constituents in aconite, although ATN has been believed to be the main active component despite its severe toxicity.

The isolated phrenic nerve-diaphragm muscles in male ddY mice (28–39 g) were used. The twitch tensions were elicited by direct and indirect stimulation (0.2 Hz, supramaximal voltage) through bipolar platinum electrodes and recorded isometrically as previously reported (12). Inhibition percentage of twitch responses to various constituents were determined at 60 min after bath (5 ml) application of the constituents. Aconite constituents are classified chemically as...
ATN-(I), chasmanine-(II), atisine-(III), CRN-(IV), HGA-(V) and the other-(VI) types as shown in Fig. 1. The delcosine-type is also among the classified constituents, but was not used. All alkaloids used were the HCl or HBr salts (Fig. 1). The HCl-salts of lipo-hypaconitine (LHA), lipodeoxyaconitine (LDA) and lipoaconitine (LAT) were solubilized in 0.0004–0.02% ethanol. Aconite extract was prepared from processed aconite (paofu) as previously reported (1).

Aconite extract (200 μg/ml) blocked the nerve-evoked twitching without affecting the muscle-evoked twitching, and the blocking action was not reversed by neostigmine (15 μM) as shown in Fig. 2A. HAT, ATN and mesaconitine (MAT) at the concentration of 2 μM behaved in the same manner as the aconite extract.

Hypaconitine (HAT, IC50: 72.3 ng/ml) most effectively inhibited the phrenic nerve-diaphragm muscle as shown in Fig. 2B; and it was 4-fold more potent than ATN (IC50: 260 ng/ml) and MAT (IC50: 287 ng/ml) and 720-fold more potent than the aconite extract (IC50: 52 μg/ml). The IC50 of deoxyaconitine (DAT) was 1.39 μg/ml. Among the constituents of processed aconite, LHA (IC50: 3.74 μg/ml) was more effective than LDA, LAT and benzoylemesaconine (BMA) (IC50: 19.5, 58.1 and 72.6 μg/ml, respectively). Among the lipo-alkaloids tested, LHA was the most potent for neuromuscular blocking activity. The IC50 of CRN was 5.19 μg/ml. Also, d/-HGA had a weakly effective (IC50: 260 μg/ml) neuromuscular blocking action, although it has potent cardiotonic action (8). Kobusine and chasmanine, the low toxicity constituents, showed much lower inhibitory effects (IC50: 520 and 564 μg/ml, respectively).

ATN depolarizes the nerve membrane after persistently activating voltage-sensitive Na+ channels (13), followed by causing an inexcitability of nerve cells (14) and causing the direct blocking of the Na+ channels of the nerve membrane (15). However, the mechanisms of the neuromuscular blocking effect by HAT has not been studied. The mode of action of HAT appears to follow the same pattern as that of ATN. As the results of the blockade of nerve excitability, acetylcholine release from nerve endings may be blocked by HAT. Recently, the differences of the sodium channels between the nerve and muscle have been reported (16). In the present study, HAT blocked the nerve-evoked twitching without affecting the directly stimulated twitching. It remains to be determined whether HAT may block only the nerve-Na+ channel.

The various pharmacological activities of HAT have been reported to be weaker than those of ATN and MAT (3). Its neuromuscular...
blocking action, however, was more potent than those of ATN or MAT. The contents of ATN and MAT in aconite root are reduced by processing with heat, but the content of HAT was not changed (4) and was determined to be 3.4–8.5-fold higher than those of ATN or

Fig. 2. Neuromuscular blocking actions of aconite extract and its constituents on mouse phrenic nerve-diaphragm muscle preparation in vitro. A: Typical recordings of isometric twitching by alternating direct and indirect stimulation (supramaximal, 0.2 Hz, 1 msec) are shown. B: The inhibition percentage of nerve-evoked twitching is plotted against log concentration (g/ml) of each constituent. The values are means±S.E.M. (n=3–10).
MAT in processed aconite (11). This means that the processing of aconite is useful in therapy, because HAT is most potent (4-fold than ATN or MAT) among the aconite constituents, and it has low toxicity (one third to one sixth the toxicities of ATN or MAT) (3).

In conclusion, the dominant constituent for the neuromuscular blocking action of aconite is HAT, and not ATN.

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References