Equipotency of Anti-Curare Activity of 4-Aminopyridine and 3,4-Diaminopyridine in Anesthetized Rats

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Abstract—In the curarized preparation, 3,4-diaminopyridine (3,4-DAP) and 4-aminopyridine (4-AP) were equiactive in their ability to antagonize d-tubocurarine caused complete depression of the indirectly elicited twitches of the sciatic nerve-tibialis anterior muscle preparation in anesthetized rats. In the non-curarized preparation, 3,4-DAP showed 2.3 to 4.0 times stronger augmentation of the indirectly elicited twitches than 4-AP, but both the drugs increased equivalently and slightly the maximally elicited twitches of the chronically denervated muscle. The results suggest that the difference of their prejunctional effects is masked by the post-junctional effects of d-tubocurarine in the indirectly elicited twitches.

It is well known that aminopyridines can enhance the stimulus-evoked release of neurotransmitter not only at neuromuscular junctions but at the other chemical synapses (1, 2). 3,4-Diaminopyridine (3,4-DAP) is about 6 to 7 times more potent than 4-aminopyridine (4-AP) in increasing transmitter release in magnesium-depressed end-plates of mice in vitro (3). The aim of this study was to examine whether 3,4-DAP would be more potent than 4-AP in vivo as it is in vitro and to determine if the difference of their prejunctional effects in vivo could be influenced by d-tubocurarine (d-Tc) mainly in non-curarized and curarized neuromuscular junctions in anesthetized rats.

The left sciatic nerve of male Wistar rats (80–90 g) was cut in about 5 mm length and removed under anesthesia with thiopental sodium (40 mg/kg, i.p.). Three weeks after the denervation, the rats (250–300 g) anesthetized with urethane (1.3 g/kg, i.p.) were supinely placed on a fixing board. Mean arterial pressure (MAP) was recorded through left common carotid arterial cannula connected to a pressure transducer. Heart rate (HR) was recorded through a electrograph connected to a tachometer. Respiratory amplitude (RA) and rate (RR) were recorded through a tracheal cannula connected to a thermometer. Indirectly elicited twitches were obtained by supramaximal rectangular pulse stimulation (0.03 msec, 0.5 Hz) of the nerve in the sciatic nerve-tibialis anterior muscle preparation of a right limb. Directly elicited muscle twitches were obtained by supramaximal rectangular pulse stimulation (5 msec, 0.5 Hz) of the denervated tibialis anterior muscle of a left limb. The twitch tension was measured by the isometric method with a force-displacement transducer. A resting tension of 2 g was constantly kept throughout the experiment. All parameters were simultaneously recorded on a polygraph. Drugs were injected rapidly through the left external jugular venous cannula. Drug concentrations were adjusted with physiological saline to an injection volume of 0.05 ml/100 g. The drugs used were 4-AP (Aldrich), 3,4-DAP (Aldrich), d-Tc (Yoshitomi), urethane (Junsei-Kagaku) and thiopental sodium (Tanabe).

All results are expressed as the mean ± S.E. of the percent changes of over 5 observations. Statistical differences were examined by Student's t-test or Aspin-Welch's test. A...
D. value less than 0.05 was regarded as significant. Regression lines were calculated by the least squares method.

In the non-curarized rats (Fig. 1), the indirectly elicited twitches were augmented by both 4-AP and 3,4-DAP in a dose-dependent manner, and the significant greater augmentative effect of 3,4-DAP was about 4.0 and 2.3 times stronger than that of 4-AP at the high doses of 30 and 100 μM/kg, respectively. When the percent increase of the indirectly elicited twitches was plotted as a function of the administered dosage from 10 to 100 μM/kg, both the regression lines for 4-AP (n=21, y=32.8 logx−15.8, F=11.88, P<0.01) and 3,4-DAP (n=21, y=128.5 logx−92.7, F=54.97, P<0.01) showed significant linearity, but the regression lines were highly different. This stronger action of 3,4-DAP agrees with the data of Molgo et al. (3) that 3,4-DAP showed a 6 to 7 times stronger facilitation effect on the transmitter release than 4-AP. The difference between our data and those of Molgo et al. regarding the potency of 3,4-DAP compared with 4-AP is probably due to differences of experimental conditions; the preparation of Molgo et al. was under the magnesium-reduced release of transmitter at prejunctional sites, and in addition, other factors like differences in the animals, a difference between in vitro and vivo conditions, and unknown factors may contribute to the difference in potency. As the indirect twitches were supramaximally produced, the augmentative effect of both 4-AP and 3,4-DAP can be readily explained to a large extent by their increasing actions on the probability of release close to the maximal level (3, 4) and by their weak augmentative effect on the directly elicited twitches as shown in this study to a much lesser extent (5, 6). About half the cases injected with 4-AP (3, 10 and 30 μM/kg) or 3,4-DAP (10 and 30 μM/kg) showed the weak augmentation of the directly elicited twitches. At a dose of 100 μM/kg, 4-AP and 3,4-DAP not only increased the twitch tension in 2 cases out of 7 and in 2 cases out of 9, respectively, but decreased it in 2 cases out of 8 and in 4 cases out of 9, respectively. The opposite increasing and decreasing effects of 4-AP or 3,4-DAP on the directly elicited twitches might be explained by the preparation conditions in which activation of the contractile mechanism is submaximal (6).
in the increased cases or by their effects on
the muscular circulation in the decreased
cases. MAP was dose-dependently increased
by the doses of 4-AP and 3,4-DAP up to
30 μM/kg which induced the maximal
response. There was no significant difference
between 4-AP and 3,4-DAP at any dose of
MAP as in the directly elicited twitches. HR
was decreased equivalently by 4-AP and
3,4-DAP (30 and 100 μM/kg). At 3 μM/kg,
3,4-DAP decreased HR significantly more
than 4-AP. Although 4-AP induced respira-
tory acceleration in a dose-dependent manner
more strongly than 3,4-DAP, the acceleration
was very variable. This difference between
4-AP and 3,4-DAP and this variability are
probably due to their different abilities to
penetrate into the central nervous system (7).

In the curarized rats (Fig. 2), 50 μM/kg
d-Tc, i.v., a dose which completely depressed
the indirectly elicited twitches for about 10
min, did not produce any significant effects
on the directly elicited twitches. HR and
MAP, but decreased RA and RR by 40.9±5.9
and 25.9±1.1%, respectively. At 30 sec after
the complete depression of the indirectly
elicited twitches with a bolus injection of
d-Tc. 4-AP or 3,4-DAP was intravenously
administered. 4-AP and 3,4-DAP dose-
dependently and equivalently reversed the
depressed twitches, except at a dose of
10 μM/kg, the ability of 4-AP to cause
reversal was significantly greater than that of
3,4-DAP (121.3±3.9 and 104.0±2.7%,
respectively). Equivalent significant linear
regression was obtained between the
reversibility of the indirectly elicited twitches
and the administered dosage of 4-AP (n=20,
y=60.7 logx+63.5, F=89.63, P<0.001) or
3,4-DAP (n=20, y=60.2 logx+58.2,
F=124.45, P<0.001). Our data demon-
strating the equipotency of 4-AP and 3,4-
DAP in their antagonistic effect on the d-Tc-
induced complete depression of the indirectly

![Fig. 2. Antagonistic action of 4-AP and 3,4-DAP (i.v.) to the d-Tc (50 μM/kg, i.v.)-induced complete
depression of the indirectly elicited twitches of the sciatic nerve-tibialis anterior muscle preparation, and
their effects on the directly elicited twitches, heart rate (HR), mean arterial pressure (MAP) and respiratory
rate (RR) and amplitude (RA) in anesthetized rats. In each group, 5 rats were used. The results are
given as the mean±S.E. The letters at the bottoms of the columns except the indirect twitch tension and
the letters with a dash beside the tops of the columns show the significant difference from the data of
d-Tc without aminopyridines and the data of 4-AP, respectively, and the letters at the bottoms of the
columns in the indirect twitch tension show the significant difference from the data of 0.1 μM/kg of
4-AP or 3,4-DAP: a,a'(P<0.05), b,b'(P<0.02), c,c'(P<0.01), d,d'(P<0.005), e(P<0.001).]
elicited twitches agree with the data of Durant et al. (8) showing that 3,4-DAP and 4-AP were equiactive in their anti-curare activity. The directly elicited twitches and MAP slightly depressed by d-Tc were dose-dependent and significantly (10 and 100 nM/kg) not only reversed but increased by 4-AP and 3,4-DAP. The highly depressed RR was significantly reversed by 4-AP (10 and 100 nM/kg) not only reversed but increased by 4-AP and 3,4-DAP. The highly depressed RR was significantly reversed by 4-AP (10 and 100 nM/kg). On the other hand, 3,4-DAP (10 and 100 nM/kg) significantly reversed the depressed RA. These data of the respiration can be explained by the higher penetration of 4-AP into brain (7) and the stronger transmitter releasing action of 3,4-DAP at the neuromuscular junction (3). No difference between 4-AP and 3,4-DAP was observed in their effect on the directly and indirectly elicited twitches, HR and MAP in the curarized rats.

Although 3,4-DAP was 2.3 to 4.0 times more potent than 4-AP in the augmentation of the indirectly elicited twitches in the non-curarized rats, 3,4-DAP and 4-AP were equiactive in their antagonism to the complete depression of the indirectly elicited twitches with d-Tc in spite of the difference in the potencies of their presynaptic effects (3). From our present data, we cannot elucidate the mechanism by which the significant difference of 3,4-DAP and 4-AP in the indirectly elicited twitches of the non-curarized muscle disappeared in those of the curarized muscle. However, Durant et al. (8) reported no difference of 3,4-DAP and 4-AP in their antagonism to the complete depression (80%) of the indirectly elicited twitches with d-Tc in spite of the difference in the potencies of their presynaptic effects (3). From our recent unpublished data, 3,4-DAP showed 2.3 times stronger antagonism than 4-AP to the slight depression of the indirectly elicited twitches by a continuous infusion of d-Tc, and in our recent unpublished data, 3,4-DAP showed 2.3 times stronger antagonism than 4-AP to the slight depression of the indirectly elicited twitches by a continuous infusion of d-Tc, and in our recent unpublished data, 3,4-DAP showed 2.3 times stronger antagonism than 4-AP to the slight depression of the indirectly elicited twitches by a continuous infusion of d-Tc, and in our recent unpublished data, 3,4-DAP showed 2.3 times stronger antagonism than 4-AP to the slight depression of the indirectly elicited twitches by a continuous infusion of d-Tc, and in our recent unpublished data, 3,4-DAP showed 2.3 times stronger antagonism than 4-AP to the slight depression of the indirectly elicited twitches by a continuous infusion of d-Tc, and in our recent unpublished data, 3,4-DAP showed 2.3 times stronger antagonism than 4-AP to the slight depression of the indirectly elicited twitches by a continuous infusion of d-Tc. Consequently, when the indirectly elicited twitches are highly depressed by d-Tc, it can be considered that the significant difference in the transmitter releasing potency of these drugs in the indirectly elicited twitches may be masked by the postjuctional adequate depression with d-Tc. In conclusion, it can be proposed that 3,4-DAP which is about 2 times less convulsive and toxic than 4-AP (9, 10) might have some advantage over 4-AP only when the release of transmitter is submaximal or reduced by drugs like aminoglycoside antibiotics (11, 12).

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