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Burst firing of action potentials in central snail neurons elicited by *d*-amphetamine: effect of anticonvulsants

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Abstract

The effect of anticonvulsants on the burst firing of action potentials in snail central neuron elicited by *d*-amphetamine was studied in the identified RP4 neuron of the African snail, *Achatina fulica* Ferussac. Oscillation of membrane potential and burst firing of action potentials were elicited by *d*-amphetamine in a concentration-dependent manner. Voltage clamped studies revealed that *d*-amphetamine elicited a negative slope resistance (NSR) in steady-state $I-V$ curve between -40 and -10 mV. The burst firing of action potentials was alleviated following extracellular application of phenytoin, but was not affected after ethosuximide, carbamazepine, and valproic acid. The NSR elicited by *d*-amphetamine was blocked by phenytoin. However, the NSR was not altered if carbamazepine was added. These results suggest that of the four anticonvulsants tested, only phenytoin could alleviate the burst firing of action potentials elicited by *d*-amphetamine in snail neuron.

Keywords: Amphetamine; Anticonvulsant; Drug abuse; Neuron; Bursting; Potential; Invertebrate; Phenytoin; Epilepsy; Comparative pharmacology

1. Introduction

Convulsant such as pentylenetetrazole induced bursting activity of action potentials in the central neurons of snails. Factors altering the effects were well studied (Sugaya et al., 1985a; Onozuka et al., 1986; Tsai and Chen, 1989; Wiemann et al., 1996). Both *d*- and *l*-amphetamines also elicited burst firing of action potentials in a central neuron located on the right parietal ganglion (RP4 neuron) of the African snail, *Achatina fulica* Ferussac (Tsai and Chen, 1995; Huang et al., 1999) and in the central thalamic neurons of new born rats (Tsai et al., 2000). The molecular mechanism underlying pentylenetetrazole-induced bursting activity is well studied (Onozuka et al., 1983, 1986, 1991a; Sugaya et al., 1985a,b). Factors altering the burst firing of action potentials elicited by d-amphetamine were tested. The effect was not blocked by high magnesium media, a number of drugs including propranolol, prazosin, haloperidol, phenobarbital, hexamethonium, *d*-tubocurarine, atropine, calcium-free solutions, or verapamil (Tsai and Chen, 1995). The bursting activity elicited by *d*-amphetamine is not due to:

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(a) the synaptic effects of neurotransmitters; or (b) the activity of cholinergic or adrenergic receptors of the excitable membrane (3). However, it has been associated with intracellular calcium ions (Chen and Tsai, 1996; Chen et al., 1998), second messengers (Chen and Tsai, 1997), and ionic currents (Chen and Tsai, 2000) of the neuron. *d*-Amphetamine elicits a negative slope resistance of the steady state $I-V$ curve (NSR) in the neuron (Chen and Tsai, 2000). The NSR is closely associated with the burst firing of action potentials in the neurons (Gillette, 1983; Funase, 1990; Onozuka et al., 1991b). Anticonvulsant drug affected on the spontaneous thalamocortical rhythm and it represented a potentially valuable in vitro model of generalized seizure discharges, with marked pharmacological and physiological similarities to various forms of clinical epileptic seizure activity (Zhang et al., 1996a,b,c). However, which anticonvulsants can alleviate the burst firing of action potentials elicited by *d*-amphetamine remained unknown. The aims of the present study were to assess the effects of anticonvulsants, i.e. phenytoin, ethosuximide, carbamazepine and valproic acid, on the burst firing of action potentials elicited by *d*-amphetamine. The results revealed that only phenytoin alleviated the burst firing of action potentials elicited by *d*-amphetamine.

2. Methods

2.1. Electrophysiological recordings

Experiments were performed on the identified RP4 neuron from the subesophageal ganglia of the African snail *Achatina fulica* Ferussac (Tsai and Chen, 1995). The ganglia were pinned to the bottom of 0.7 ml sylgar-coated perfusion chamber and the connective tissue sheath was removed to allow easy identification and penetration by microelectrodes. For voltage-clamp study, two microelectrodes were penetrated into the neuron. The recording electrode (5–6 MΩ) and the current electrode (1–5 MΩ) were filled with 3 M KCl. The experimental chamber was perfused with a control saline. Solution composed of (mM): NaCl 85, KCl 4.0, $CaCl_2$ 8, $MgCl_2$ 7, Tris–HCl 10 (pH 7.5) at room temperature of 23–24°C. Neurons were studied only if they exhibited resting membrane potentials more negative than -50 mV (4). The ionic currents of the RP4 neurons were recorded using two-electrode voltage-clamp method. For voltage clamping, the neurons were clamped by means of Gene Clamp 500 amplifier (Axon Instrument). All potentials and currents were recorded on magnetic tape via a digitizing unit (Digidata 1200) and analyzed using the pCLAMP system. The steady state currents of the neurons were measured at the end of 5 s pulses (Chen and Tsai, 2000).

Data obtained after various treatments were compared with the pre-drug control by means of Student's two-tailed t-test and paired t-test. Student's t-test was used when the samples in the control and experimental conditions were from different groups of preparations. Student's paired t-test was used when the samples in the control and experimental condition were from the same groups of preparations. Differences were considered significant at $P < 0.05$. Phenytoin (5,5-diphenylhydantoin sodium salt), carbamazepine, valproic acid, ethosuximide and *d*-amphetamine were purchased from Sigma (St Louis, MO).

3. Results

3.1. Electrophysiological properties of RP4 neurons

The resting membrane potential (RMP) of the identified RP4 neuron was -60.1 ± 0.6 mV ($n = 15$, mean $\pm$ S.E.M.) and it showed a spontaneous firing of action potential at a frequency of 37.4 ± 1.5 pulses/min ($n = 15$). The action potential showed a regularly spaced single spike. No burst firing of action potential was observed in control RP4 neuron. The mean amplitude of the spontaneously generated action potentials was 83.2 ± 0.8 mV ($n = 15$). The electrical characteristics of the RP4 neuron were quite similar to those reported previously (Tsai and Chen, 1995).

3.2. Effects of d-amphetamine on spontaneous action potentials of RP4 neurons

The identified RP4 neuron showed a prominent response to *d*-amphetamine. Twenty minutes after extracellular perfusion of *d*-amphetamine (27 μM), the frequency of spontaneously firing action potentials was reduced. Higher concentration of

d-amphetamine, e.g. 80 μM, farther decreased the frequency of the action potentials. No burst firing of action potential was observed even after 4 h of application. However, 20 min after application of 270 μM, 94 neurons (out of 120 tested) showed the firing pattern changed from regularly spaced single spikes to one with bursts of 2–40 action potentials which were separated by a period of hyperpolarization. Oscillation of membrane potentials with a phasic depolarization followed by a sustained depolarization with burst of action potentials was found in *d*-amphetamine (270 μM) treated preparations. A sudden depolarization with a train of spikes on its rising phase was observed. Furthermore, 40 min after application of *d*-amphetamine (270 μM), the bursting activity was found in 117 out of 120 neurons tested. Forty minutes after application of *d*-amphetamine (270 μM), each burst contained 10–40 action potentials and was terminated by hyperpolarization of the membrane. There were 1–5 bursts of action potentials in 1 min (3.2 ± 0.7 bursts/min, $n = 5$). The number of action potentials in each burst were in the range of 2–40 depending on individual neuron tested. However, in the same neuron, the number of action potentials in each burst were reach steady after 40 min of *d*-amphetamine administration. In five neurons calculated, the burst

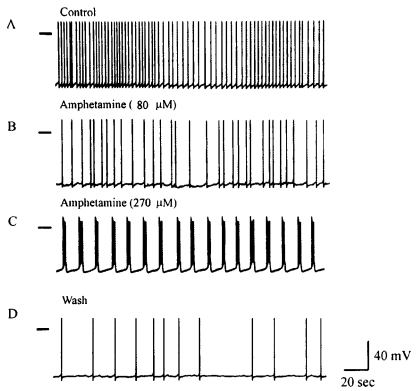

Fig. 1. Effects of *d*-amphetamine on a central RP4 neuron of snail. A, B, C and D were from the same neuron. (A) Control, the neuron showed spontaneous firing of action potentials. (B) Potentials of RP4 neuron after 40 min of *d*-amphetamine (80 μM) administration. (C) Bursting firing of action potentials of the neuron 40 min after *d*-amphetamine (270 μM) administration. (D) The potentials after 75 min washing off *d*-amphetamine (270 μM). The horizontal bar on the top left side indicated the membrane potential at 0 mV. Note that *d*-amphetamine at 80 μM did not, while *d*-amphetamine at 270 μM did, elicit burst firing of action potentials of RP4 neuron.

were 18.8 ± 0.6 pulses/burst ($n = 5$). The subtained depolarization depolarized reached a level of -39.8 ± 2.1 mV ($n = 5$). This was followed by a long fall to a hyperpolarized level (Fig. 1C). The resting membrane potentials of the neurons were changed from -60.1 ± 0.6 mV ($n = 15$) to -67.4 ± 0.9 mV ($n = 5$) during the hyperpolarization. There was no single-spike action potential 40 min after *d*-amphetamine (270 μM) treatment. The effect of *d*-amphetamine on the neuronal activities was reversible. After 120 min of continuous washing, the spontaneously generated spikes of the central neuron returned to control level albeit with a lower frequency of spontaneous firing. An example of the effects of *d*-amphetamine on the action potentials of the snail neuron is shown in Fig. 1.

3.3. Effects of phenytoin on the d-amphetamine elicited bursting activity of RP4 neuron

The effects of phenytoin on the *d*-amphetamine-elicited bursting activity of RP4 neuron are shown in Figs. 2A and 3A. At 10 μM, phenytoin significantly decreased the number of action potentials during the burst elicited by *d*-amphetamine. The number of action potentials in the presence of *d*-amphetamine (270 μM) and 30 min after perfusion with *d*-amphetamine containing phenytoin (10 μM) were 18.8 ± 0.6 pulse/burst and 2.6 ± 0.4 pulse/burst ($n = 5$, $P < 0.05$), respectively. Spaced single spikes were at the rate of 4.4 ± 0.5 pulses/min were observed when *d*-amphetamine (270 μM) and phenytoin (10 μM) were co-administered. At higher concentration, phenytoin (50 μM) significantly decreased (a) the frequency of burst firing of action potentials and (b) the number of action potentials during the burst elicited by *d*-amphetamine. The frequency of bursting activity in the presence of *d*-amphetamine (270 μM) and 30 min after perfusion with *d*-amphetamine containing phenytoin (50 μM) was 3.2 ± 0.7 and 1.2 ± 0.2 burst/min ($n = 5$, $P < 0.05$), respectively, and the number of action potentials in each burst was 18.8 ± 0.6 and 2.2 ± 0.2 pulse/burst ($n = 5$, $P < 0.05$), respectively. The number of spaced single-spike potentials increased to 11.4 ± 1.1 pulse/min. However, bursting activity still appeared in phenytoin treated preparation although most of the action potentials changed its pattern to the spaced single spike (Figs. 2A and 3A). The effect of phenytoin on the burst firing of

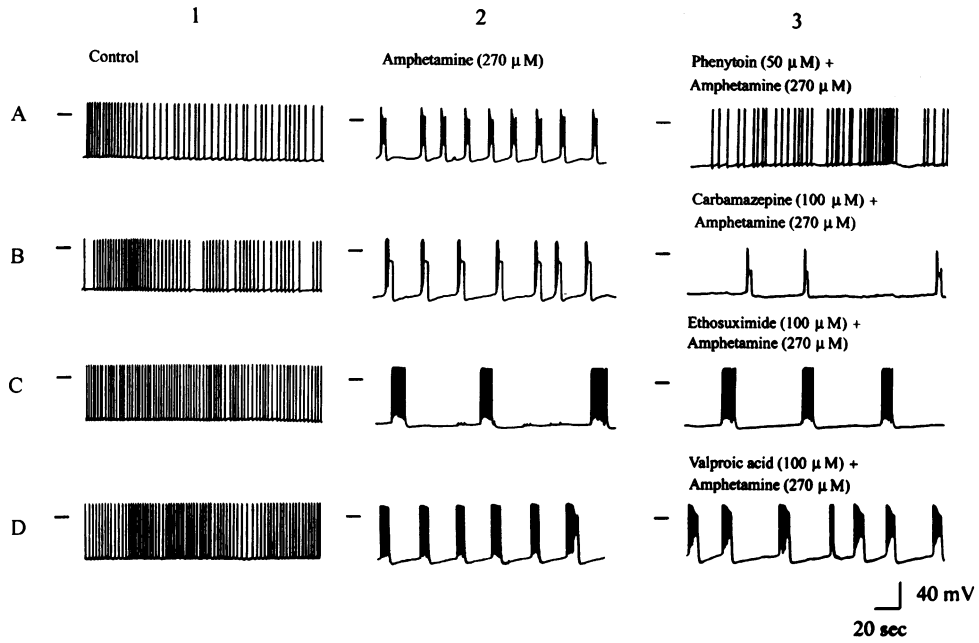

Fig. 2. Effects of phenytoin, carbamazepine, ethosuximide and valporic acid on the *d*-amphetamine elicited burst firing of action potentials in RP4 neuron. A, B, C and D were from different neurons. A1, B1, C1 and D1: Controls, the spontaneous action potentials from four RP4 neurons, respectively. A2, B2, C2 and D2: The burst firing of action potentials 40 min after perfusion with *d*-amphetamine (270 μM) from A1, B1, C1 and D1, respectively. A3, B3, C3 and D3: Thirty minutes after further perfusion with *d*-amphetamine (270 μM) containing phenytoin (50 μM, A3), carbamazepine (100 μM, B3), ethosuximide (100 μM, C3) and valproic acid (100 μM, D3) from A2, B2, C2 and D2, respectively. The horizontal bar on the top left side indicated the membrane potential at 0 mV. Note that the pattern of burst firing of action potentials was alleviated following extracellular application of phenytoin and the burst firing of potentials was changed into regularly spaced single spikes. The pattern of burst firing of action potentials was found in *d*-amphetamine with ethosuximide, carbamazepine and valproic acid treated preparations.

action potentials elicited by *d*-amphetamine was reversible. Thirty minutes after washing off phenytoin with *d*-amphetamine, the bursting activity resumed. Thus, phenytoin (50 μM) reversibly alleviated the bursting firing of action potentials elicited by amphetamine in a concentration dependent manner.

3.4. Effects of phenytoin on the spontaneously firing action potentials of RP4 neuron

The RMP, the frequency and amplitude of the action potentials in control RP4 neuron were -60.1 ± 0.6 mV ($n = 15$), 37.4 ± 1.5 pulses/min ($n = 15$) and 83.2 ± 0.8 mV ($n = 15$), respectively. Thirty minutes after phenytoin (10 μM) perfusion, the RMP and the frequency and amplitude of the action potentials were -60.3 ± 0.9 mV ($n = 3$, $P > 0.05$) 39.6 ± 0.4 pulses/min ($n = 3$, $P > 0.05$) and 84.3 ± 0.3 mV ($n = 3$, $P > 0.05$), respectively. Thirty minutes after phenytoin (50 μM) perfusion, the RMP and the frequency and amplitude of the action potentials were -59.3 ± 1.5 mV ($n = 3$, $P > 0.05$) 39.3 ± 1.6 pulses/min ($n = 3$, $P > 0.05$) and 83.6 ± 0.9 mV ($n = 3$, $P > 0.05$), respectively. It appeared that phenytoin (10–50 μM) did not alter the RMP, the frequency and amplitude of the action potentials of the RP4 neuron.

3.5. Effects of carbamazepine on the d-amphetamine elicited bursting activity of RP4 neuron

Forty minutes after perfusion with *d*-amphetamine (270 μM), the bursting firing of action potentials was elicited in RP4 neuron. The pattern of bursting activity did not change and there was no single spike action potentials 30 min after perfusion with carbamazepine (100 μM) containing *d*-amphetamine (270 μm). However, the frequency of bursting in the presence of *d*-amphetamine and 30 min after perfusion with carbamazepine (100 μM) containing *d*-am-

phetamine (270 μM) were 2.6 ± 0.3 burst/min and 0.6 ± 0.3 burst/min ($n = 3$, $P < 0.05$), respectively. Similar results were found in three other preparations. Examples of the effects of carbamazepine on *d*-amphetamine elicited bursting firing of action potentials were shown in Figs. 2B and 3B. It appears that carbamazepine (100 μM) did not alter the pattern of bursting firing of action potentials elicited by *d*-amphetamine.

3.6. Effects of ethosuximide on the d-amphetamine elicited bursting activity of RP4 neuron

Forty minutes after perfusion with *d*-amphetamine (270 μM), the bursting firing of action potentials was elicited in the RP4 neuron (Figs. 2C and 3C). The pattern of bursting activity did not change and there was no single spike action potentials 30 min after perfusion with ethosuximide (100 μM) containing *d*-amphetamine (270 μM). Similar results were found in another three neurons. It appears that ethosuximide (100 μM) did not alter the pattern of bursting firing of action potentials elicited by *d*-amphetamine.

3.7. Effects of valproic acid on the d-amphetamine elicited bursting activity of RP4 neuron

Forty minutes after perfusion with *d*-amphetamine (270 μM), the bursting firing of action potentials was elicited in the RP4 neuron. The pattern of bursting activity did not change and there was no single spike action potentials 30 min after perfusion with valproic acid (100 μM) containing *d*-amphetamine (270 μM). Similar results were found in five other preparations. Examples of the effects valproic acid on the *d*-amphetamine elicited bursting firing of action potentials were shown in Figs. 2D and 3D. It appears that valproic acid (100 μM) did not affect the pattern of bursting firing of action potentials elicited by *d*-amphetamine.

3.8. The steady-state currents of RP4 neuron

Currents in response to test potentials of -50, -30, -10 and 10 mV are shown in Figs. 4.1 and 5.1. The currents were elicited by 5 s command pulses from a holding potential of -60 mV to potentials ranging from -100 to 10 mV. The

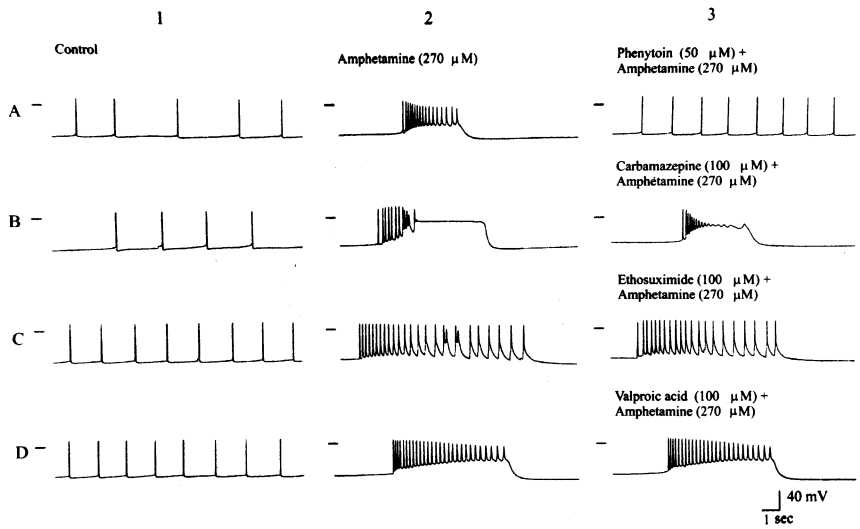

Fig. 3. The expanded pictures showing individual action potentials related to the effects of phenytoin, carbamazepine, ethosuximide and valporic acid on the *d*-amphetamine elicited burst firing of action potentials in RP4 neuron. A, B, C and D were from different neurons. A1, B1, C1 and D1: Controls, the spontaneous action potentials from four RP4 neurons, respectively. A2, B2, C2 and D2: The burst firing of action potentials 40 min after perfusion with *d*-amphetamine (270 μM) from A1, B1, C1 and D1, respectively. A3, B3, C3 and D3: Thirty minutes after further perfusion with *d*-amphetamine (270 μM) containing phenytoin (50 μM, A3), carbamazepine (100 μM, B3), ethosuximide (100 μM, C3) and valproic acid (100 μM, D3) from A2, B2, C2 and D2, respectively. The horizontal bar on the top left side indicated the membrane potential at 0 mV. Note that the pattern of burst firing of action potentials was alleviated following extracellular application of phenytoin and the burst firing of potentials was changed into regularly spaced single spikes. The pattern of burst firing of action potentials was found in *d*-amphetamine with ethosuximide, carbamazepine and valproic acid treated preparations.

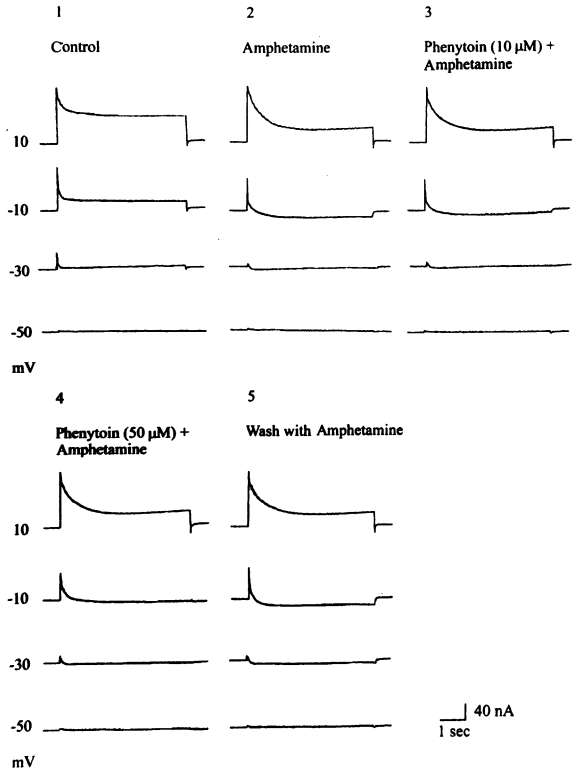

Fig. 4. Effects of phenytoin on the *d*-amphetamine-elicited current changes in RP4 neuron. Steady-state currents were elicited by 5 s command steps from holding potentials of −60 mV to stepping potentials of −50, −30, −10 and 10 mV. (1) Control. The neuron was perfused with normal physiological saline. (2) Forty minutes after subsequent *d*-amphetamine (270 μM) administration. (3) Thirty minutes after phenytoin (10 μM) and *d*-amphetamine (270 μM) was subsequently perfused. (4) Phenytoin (50 μM) and *d*-amphetamine (270 μM) was subsequently perfused. (5) Thirty minutes after washing off with *d*-amphetamine (270 μM).

steady-state current–voltage (I–V) relationships are shown in Fig. 6. The steady-state outward currents were observed with pulse potentials more positive than −50 mV. The total outward current increased with more positive potential (Chen and Tsai, 2000).

3.9. Effect of d-amphetamine on the steady-state currents of RP4 neuron

The effects of *d*-amphetamine on the steady-state currents of RP4 neuron are shown in Figs. 4 and 5. Compared with pre-drug controls, the steady-state currents of the neurons were significantly decreased 40 min after *d*-amphetamine (270 μM) treatment at potentials more positive than −50 mV. The steady-state outward currents reversed its polarity into inward currents and the steady-state I–V curve revealed an N-shaped appearance in the range from −40 to −10 mV after *d*-amphetamine (270 μM) treatment (6) (Figs. 4–6). The phenomenon revealed as the NSR of the steady state I–V curve. Both the burst firing of action potentials and NSR elicited by *d*-amphetamine were found 40 min after drug administration. The effects of *d*-amphetamine on the NSR response were reversible. After washing off *d*-amphetamine (270 μM) for 120 min, the outward steady-state membrane currents were returned with no NSR in the steady state I–V curve.

3.10. Effects of phenytoin on the d-amphetamine-elicited steady-state currents

The effects of phenytoin on the *d*-amphetamine-elicited steady-state currents changes and the I–V relationships are shown in Figs. 4 and 6A and summarized in Table 1. Forty minutes after *d*-amphetamine (270 μM) treatment, NSR of the steady-state I–V curve was found in potentials of −40 to −10 mV. Thirty minutes after further perfusion with phenytoin (10 μM) containing *d*-amphetamine (270 μM), the outward currents during steps to −30 and −10 mV were

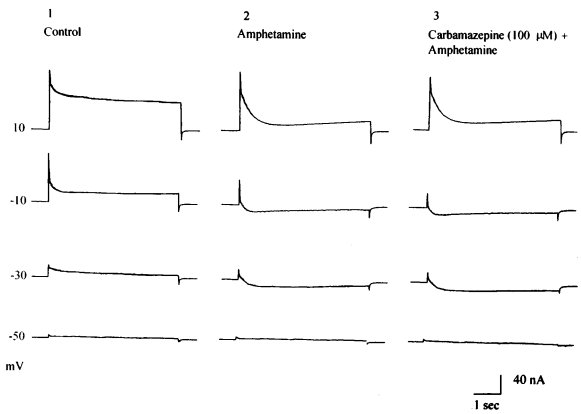

Fig. 5. Effects of carbamazepine on the *d*-amphetamine-elicited current changes in RP4 neuron. Steady-state currents were elicited by 5 s command steps from holding potentials of −60 mV to stepping potentials of −50, −30, −10 and 10 mV. (1) Control. The neuron was perfused with normal physiological saline. (2) Forty minutes after subsequent *d*-amphetamine (270 μM) administration. (3) Thirty minutes after carbamazepine (100 μM) and *d*-amphetamine (270 μM) was subsequently perfused.

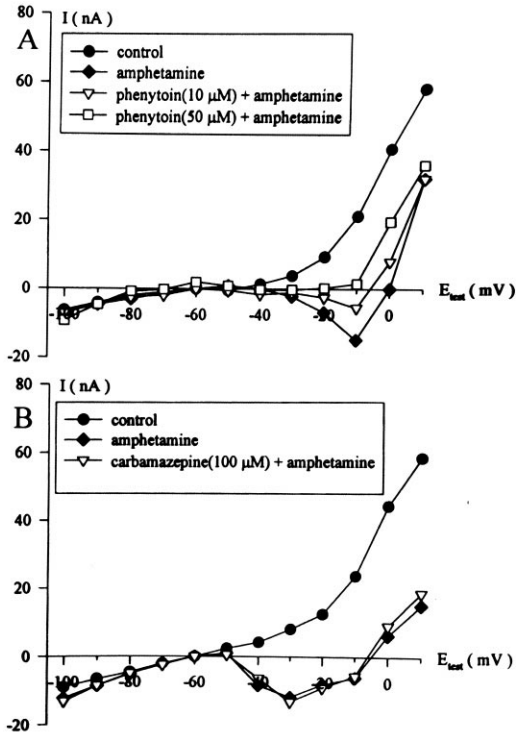

Fig. 6. (A) Effects of phenytoin on the *d*-amphetamine-elicited negative slope resistance (NSR) of the steady-state *I–V* curve. The currents were elicited by 5 s command steps from holding a potential of −60 mV to stepping potentials ranging from −100 to 10 mV at intervals of 10 mV. The steady-state currents were measured 5 s after initiation of stepping potentials. The closed circle (●), closed diamond (◆), open triangle (▽) and open square (□) points represented the steady-state *I–V* relationship before (●), after *d*-amphetamine (270 μM) application (◆), after 30 min further perfusion with phenytoin (10 μM) and *d*-amphetamine (270 μM) (△) and 30 min after further perfusion with phenytoin (50 μM) and *d*-amphetamine (270 μM) (□). Note that *d*-amphetamine (270 μM) elicited a NSR in the steady-state current–voltage relationships, which was reduced by phenytoin (10 and 50 μM). (B) Effects of carbamazepine on the *d*-amphetamine-elicited NSR of the steady-state *I–V* curve. The closed circle (●), closed diamond (◆) and open triangle (▽) points represented the steady-state *I–V* relationship before (●), after *d*-amphetamine (270 μM) application (◆), after 30 further perfusion with carbamazepine (100 μM) and *d*-amphetamine (270 μM). Note that *d*-amphetamine (270 μM) elicited a NSR in the steady-state current–voltage relationships, and it was not affected by carbamazepine (100 μM). Note that after *d*-amphetamine administration, the steady state currents elicited by depolarizing command steps in the range of −50–10 mV became more inward than those in the control. The effects of *d*-amphetamine were partially reversed if phenytoin (10 μM) was perfused and were further reversed if phenytoin (50 μM) was perfused. However, the NSR was not altered in carbamazepine (100 μM) perfused preparations.

increased (Fig. 4 and Table 1). The NSR of the steady-state *I–V* curve was decreased (Fig. 6A). Thirty minutes after further perfusion with a higher concentration of phenytoin (50 μM) containing *d*-amphetamine (270 μM), the outward currents during steps to −30 and −10 mV were further increased (Fig. 4 and Table 1). The NSR of the steady-state *I–V* curve was further decreased (Fig. 6A, Table 1). The NSR of the steady-state *I–V* curve returned after phenytoin was washed off for 30 min.

It appears that extracellular perfusion of phenytoin decreased the NSR of the steady state *I–V* curve elicited by *d*-amphetamine in a concentration dependent manner.

3.11. Effects of carbamazepine on the d-amphetamine-elicited steady-state currents

The effects of carbamazepine on the *d*-amphetamine-elicited steady-state currents changes and the *I–V* relationships are shown in Figs. 5 and 6B. Forty minutes after damphetamine (270 μM) treatment, NSR of the steady-state *I–V* curve appeared in potentials of −40 to −10 mV. Thirty minutes after further perfusion with carbamazepine (100 μM) containing *d*-amphetamine (270 μM), the outward currents during steps to −30 and −10 mV did not significantly change compared to the currents in *d*-amphetamine (270 μM) alone (Figs. 5 and 6B). It appears that extracellular perfusion of carbamazepine did not affect the NSR of the steady-state *I–V* curve elicited by *d*-amphetamine.

4. Discussion

Convulsant such as pentylenetetrazole induced bursting activity of action potentials in the central neurons of snails (Sugaya et al., 1985b; Tsai and Chen, 1989; Wiemann et al., 1996). Both d- and *l*-amphetamines also elicited burst firing of action potentials in the central RP4 neuron of the African snail, *A. fulica* Ferussac (Tsai and Chen, 1995; Huang et al., 1999). However, pentylenetetrazole elicited bursting firing of potentials in most of the central neurons in the giant African snail (Tsai and Chen, 1989) while *d*-amphetamine elicited only to some specific neurons, such as RP4 neuron of the African snail (Tsai and Chen, 1995; Huang et al., 1999). In the present study, it was found that the burst firing of action poten-

tials was alleviated following extracellular application of phenytoin, but was not affected after ethosuximide, carbamazepine, and valproic acid. A previous study also revealed that phenobarbital sodium (0.43 mM) did not alleviate the frequency and firing pattern of the central neuron elicited by *d*-amphetamine (Tsai and Chen, 1995).

Antiepileptic drugs decreased membrane excitability by interacting with neurotransmitter receptors or ion channels. Barbiturates enhanced GABAa receptor-mediated inhibition. Phenytoin and carbamazepine and possibly valproate decreased high frequency repetitive firing of action potentials by enhancing sodium channel inactivation. Ethosuximide and valproate acid reduced a low threshold (T type) calcium channel current (Macdonald and Kelly, 1995).

Phenytoin suppressed the sodium current of squid giant axon when applied internally or externally (Morello et al., 1984). It appears that phenytoin and carbamazepine possess membrane stabilization effect on squid giant axons. They blocked the sodium currents of the excitable membrane. However, in the present study, phenytoin (10–50 μM) did not alter the resting membrane potentials, the frequency and amplitude of the action potentials of the RP4 neuron. The pattern of the spontaneously generated action potential of the RP4 neuron was not altered even after 2 h of high concentration of phenytoin application. These results suggest that phenytoin did not inhibit the generation and propagation of action potentials on the excitable membrane of the RP4 neuron. The RP4 neurons possess tetrodotoxin insensitive sodium current and calcium current, and tetrodotoxin can not block the burst firing of action potentials elicited by *d*-amphetamine (Chen and Tsai, 2000). Therefore, the alleviation effect of phenytoin on the burst firing elicited by *d*-amphetamine may not mainly due to its effect on the sodium currents of the membrane.

Phenytoin and carbamazepine also potentiated γ-aminobutyric acid (GABA) induced chloride currents in human embryonic kidney cells transiently expressing the α1 β2 γ2 subtype of the $GABA_A$ receptor and in cultured rat cortical neurons (Granger et al., 1995). However, the burst firing of action potentials elicited by *d*-amphetamine was not due mainly to the activation of these receptors (Tsai and Chen, 1995). Besides, phenobarbital and carbamazepine which potentiated GABA induced chloride currents, did not alleviate the *d*-amphetamine elicited bursting firing of action potentials in RP4 neuron. Thus, the alleviation effect of phenytoin on the burst firing of action potentials elicited by *d*-amphetamine may not mainly due to the potentiation of the GABA induced chloride current.

The bursting activity elicited by *d*-amphetamine is not due to (a) the synaptic effects of neurotransmitters or (b) the activity of cholinergic or adrenergic receptors of the excitable membrane because the burst firing of action potentials elicited by *d*-amphetamine is not blocked by high magnesium media, a number of drugs including propranolol, prazosin, haloperidol, phenobarbital, hexamethonium, *d*-tubocurarine and atropine, calcium-free solutions, or verapamil (Tsai and Chen, 1995). The bursting firing of potential changes elicited by *d*-amphetamine has been asso-

Table 1
Effects of *d*-amphetamine and phenytoin on the steady-state currents of RP4 neurons[a]

	a	b	c	d
Stepping potentials (mV)	Control (nA) $n=3$	Amphetamine (nA) $n=3$	Phenytoin (10 μM)+amphetamine (nA) $n=3$	Phenytoin (50 (μM)+amphetamine (nA) $n=4$
−30	5.1 ± 1.0	−6.4 ± 1.8*	−1.1 ± 0.4**	0.1 ± 0.2***
−10	22.4 ± 1.5	−9.5 ± 2.2*	−3.1 ± 0.8**	0.6 ± 0.4***

[a] The steady-state currents were elicited by a 5-s pulse from a holding potential of −60 mV to potentials (−30 and −10 mV). The amplitudes of steady-state currents were measured at 5 s in (a) normal saline, (b) *d*-amphetamine (270 μM) treatment for 40 min, (c) further perfusion with phenytoin (10 μM) containing *d*-amphetamine (270 μM) from (b), (d) further perfusion with phenytoin (50 μM) containing *d*-amphetamine (270 μM) from (b). Values were expressed as the mean ± S.E.M. (*n*, the number of neurons tested).

* $P<0.05$, statistically significant by use of the *t*-test compared with a and b.

** $P<0.05$, statistically significant by use of the *t*-test compared with b and c.

*** $P<0.05$, statistically significant by use of the *t*-test compared with c and d.

ciated with (a) calcium ions (Chen and Tsai, 1996; Chen et al., 1998), (b) intracellular second messengers (Chen and Tsai, 1997), and (c) ionic currents (Chen and Tsai, 2000) of the neuron.

The intracellular calcium ion was associated with the action potential bursts elicited by *d*-amphetamine. The occurrences of the action potential bursts elicited by *d*-amphetamine was decreased following intracellular injection with either EGTA or magnesium ions or extracellular application of lanthanum (Chen and Tsai, 1996). The bursting firing of action potentials was also decreased following extracellular application of (1) H8 (*N*-(2-methyl-amino) ethyl-3-isoquinoline sulphonamide dihydrochloride), a specific protein kinase A inhibitor and (2) anisomycin, a protein synthesis inhibitor. However, the bursting firing of action potentials were not affected after (1) extracellular application of H7 (1,(5-isoquinoline-sulphonyl)-2 methylpiperasine dihydrochloride), a specific protein kinase C (PKC) inhibitor, or (2) intracellular application of GDPβS, a G protein inhibitor. The oscillation of membrane potential of the bursting activity was blocked after intracellular injection of 3′-deoxyadenosine, an adenylyl-cyclase inhibitor. These results suggested that the bursting firing of action potentials elicited by *d*-amphetamine in snail neuron may be associated with the cyclic AMP second messenger system; on the other hand it may not be associated with the G protein and PKC activity (Chen and Tsai, 1997). The intracellular cyclic nucleotide and calcium ion also play an important role on the pentylenetetrazole-induced bursting activity in *Euhadra* neurons (Onozuka et al., 1983; Sugaya et al., 1985a). Voltage clamp studies on the RP4 neuron revealed that *d*-amphetamine decreased the steady-state K^+ current and elicited a NSR in the steady-state $I-V$ curve between -50 and -10 mV. The amplitude of NSR was decreased if either Na^+-free saline or Co^{2+}-substituted Ca^{2+} free saline were perfused (Chen and Tsai, 2000). In the present study, it was found that phenytoin did, while carbamazepine did not, block the NSR elicited by *d*-amphetamine. Its interesting to note that there are two serotonin-sensitive potassium channels in the identified heart excitatory neuron (PON) of the same snail. The activities of both channels could be recorded in the steady state and those activities disappeared on application of serotonin (Furukawa and Kobayashi, 1988). It is likely that the inhibitory effect of phenytoin on the burst firing of action potentials elicited by *d*-amphetamine may be due to its effects on inhibition of (a) intracellular calcium concentration, (b) cAMMP activity and (c) NSR elicited by *d*-amphetamine in the neuron. It is interesting to note that pentylenetetrazole elicited bursting activity in the central neuron of the Japanese land sn4 *Euhadra peliomphala*, phenytoin inhibited the calcium release. Phenytoin also inhibited the pentylenetetrazole elicited changes in (1) the intracellular calcium binding state change near the cell membrane, (2) the intracellular protein changes induced by pentylenetetrazole and the intracellular protein and (3) the increase in calcium dependent protein kinase activity during pentylenetetrazole elicited bursting activity (Sugaya et al., 1985a,b). Phenytoin also inhibited the NSR in the bursting firing of potential changes elicited by injection of a specific 70k protein in the central neurons of snail (Onozuka et al., 1991a). Both pentylenetetrazole and amphetamine elicited bursting firing of potential changes. However, pentylenetetrazole elicited bursting firing of potentials in most of the central neurons (Tsai and Chen, 1989) while *d*-amphetamine elicited only to some specific neurons, such as RP4 neuron of the African snail, *A. fulica* Ferussac (Tsai and Chen, 1989; Huang et al., 1999). The reasons for the different effects of both compounds on the neuron remained unclear. However, the peptides in the neuron may play an important role on the function. Anisomycin, the inhibitor of protein synthesis, did not alter the resting membrane potentials of the neuron. However, anisomycin significantly decreased the frequency of the spontaneously generated action potentials of the neuron. Anisomycin pretreatment prevented the bursting firing of action potentials elicited by *d*-amphetamine (Chen and Tsai, 1997). The new peptides and neuronal signaling in the neuron after different convulsants administration remained an interesting subject for further study.

In the present study, it was found that phenytoin did (a) alleviate the burst firing of action potentials and (b) block the NSR elicited by *d*-amphetamine in snail neuron while carbamazepine did not. It is concluded that phenytoin can alleviate the burst firing of action potentials elicited by *d*-amphetamine in snail neuron. The alleviation by phenytoin on the burst firing of action potentials may be due to its effects on (a) calcium ion, (b) cAMP activity, and (b) NSR

elicited by *d*-amphetamine on the excitable membrane.

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