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Effects of Anticonvulsants on Acute and Tonic Pains in the Rat*

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=ABSTRACT=

Different neural substrates have been reported to be implicated in analgesic mechanisms in the acute phasic and the sustained tonic pains. To explore the differential antinociceptive action of diphenylhydantoin (DPH) and carbamazepine (CBZ) on the acute phasic and the tonic pains, changes in tail flick latency, hot plate latency and the formalin-induced nociceptive score were assessed prior to and after intraperitoneal administration of DPH (20 & 40 mg/Kg) and CBZ (20 mg/Kg). In 11 rats, CBZ was administered repeatedly for 6 days at the dose of 20 mg/Kg/day. Also studied were the effects of strychnine and picrotoxin (1 mg/Kg, i.p.) on the CBZ-produced changes in the formalin-induced pain behaviors. The tail flick and hot plate latencies were not changed after administration of DPH and CBZ. However, DPH strongly suppressed the formalin-induced tonic pain. A single and the repeated administration of CBZ inhibited both the early phasic and the late tonic pain responses to formalin in a similar manner. On the other hand, the antinociceptive actions of CBZ were not altered by strychnine or picrotoxin. These experimental findings lead to the conclusion that DPH and CBZ have differential antinociceptive action on the acute and the tonic pains and that their antinociceptive actions are independent of the GABA- and glycine-receptors.

Key Words: Acute and tonic pain, Differential antinociception, Anticonvulsants, Rat

INTRODUCTION

The anticonvulsants are known to interfere with the sodium mechanism involved in the genesis of the action potential with little or no changes in the resting membrane potential and the potassium currents. Electrophysiological studies have shown that diphenylhydantoin (DPH) and carbamazepine (CBZ) fairly selectively reduced the inward sodium current in a dose - dependent manner in a variety of preparations such as brain synaptosomes (Willow et al, 1984), skeletal muscle (Dwyer, 1978), squid giant axon (Morello & Begenisich, 1979), frog myelinated fiber (Courtney & Etter, 1983) and mouse neuroblastoma cells (Matsuki et al, 1984; Willow et al, 1985). It has also been demonstrated that these drugs reduced the maximal rate of rise, frequency, duration and amplitude of the action potentials while did not affect sodium influx in the unstimulated resting nerve (Pincus, 1972; Ayala et al, 1977a; Mclean & Macdonald, 1982). Inhibitory actions became more strong when membrane

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potential was held at more depolarized level and the conditioning stimulations with the higher frequencies and longer duration were applied (Matsuki et al, 1984; Willow et al, 1985). Morello and Begenisich (1979) reported that blocking action of Na channels by DPH became more strong in an acidic medium than in an alkaline one.

DPH has also been known to suppress Ca^{++} influx in the K^{+} - depolarized nerve preparations, which in turn resulted in the reduction of intracellular accumulation of calcium and the calcium-dependent action potential duration and amplitude (Sohn & Ferrendelli, 1973; 1976; Mclean & Macdonald, 1982). This inhibitory action of DPH was not observed in an undepolarized synaptosome (Sohn & Ferrendelli, 1973). The suppressive action of anticonvulsants on calcium - as well as sodium - dependent action potentials inhibits the presynaptic release of neurotransmitters such as acetylcholine (Gage et al, 1980), norepinephrine (Pincus & Lee, 1973) and others (Fichman et al, 1970; Maeherbe et al, 1972). Reduction in the action potentials and the transmitter release acts together to inhibit signal transmission at the neuromuscular junction and various synapses. Another important action of anticonvulsants is to facilitate pre- and postsynaptic inhibitory mechanisms while to inhibit excitatory mechanism. DPH selectively augment the GABA-mediated postsynaptic inhibition in the cultured mouse spinal neurons (Macdonald, 1978; Macdonald & Bergey, 1979), the crayfish stretch receptors (Ayala et al, 1977b) and the inhibitory basket interneurons of rat hippocampus (Lee et al, 1979) without any effect on the responses to glycine, alanine and glutamate (Macdonald, 1978; Macdonald & Barker, 1979). On the other hand, convulsants such as pentylenetetrazol and penicillin selectively antagonize the GABA-mediated postsynaptic inhibition (Macdonald & Barker, 1977) and increase the calcium-dependent action potential (Heyer et al, 1982), which result in an increase of neuronal excitability and may lead to sustained high-frequency repetitive firings. DPH and other anticonvulsants also have the inhibitory action on excitatory postsynaptic potential (EPSP) recorded from the guinea pig hippocampal slices (Schneiderman & Schwarzkroin, 1982) and the lamprey spinal interneurons (Selzer, 1978) and repetitive discharges induced by low calcium and potassium (Richelson & Tuttle, 1975).

In clinical experiments, CBZ and other anticonvulsants have been known to be effective for treatment of pains resulting from postsympathectomy, post-laminectomy, trigeminal neuralgia and postherpetic neuralgia (Raskin et al, 1974; Swerdlow & Cundill. 1981). Although the responses of cat WDR cells to noxious stimuli such as pinch and C fiber stimulation were greatly suppressed by the intravenously administered anticonvulsants (Kim et al, 1993), the exact mechanism of analgesic action of the anticonvulsants is not clear.

Dennis and Melzack(1979) have suggested that there are two anatomically distinct pain systems, one for brief phasic pain and the other for sustained tonic pain. The different neural substrates were reported to be involved in analgesic mechanism in these two pain models (Abbott & Melzack, 1982; Ryan et al, 1985). The present study was undertaken to examine whether DPH and CBZ have differential antinociceptive actions on the acute pain (such as tail flick and hot plate test) and also on tonic pain in the experimental animals.

METHOD

This experiment was carried on 92 Sprague-Dawley rats weighing about 150~200 gm. The antinociceptive action of DPH and CBZ was assessed by two acute and one tonic pain tests; tail flick, hot plate and formalin tests. The tail flick and hot plate latencies were measured as the time from the onset of heating the tail to withdrawal of tail from the heat and as the time from placing a rat on the pre-heated hot plate (53°C) to licking the hind

paw, respectively. In the tail flick test, a projector lamp (250W) was used as the heat source and the intensity of the heat was adjusted to give the control latency of 8～9 sec. Rats were placed in the restrainer which has a hole for tail protrusion. Heat was focused on the undersurface of the tail about 3cm from its end. If the rat did not remove their tails from the heat source within 15 sec or did not lick the hind paw within 40 sec, the trial was terminated to avoid the possible tissue damages. For the formalin test, 10 μl of 5% formalin was injected into the plantar surface of hind paw and the rat was then placed in the observation chamber which has an elevated glass floor with glass dome. A mirror was placed below the elevated floor at an angle of 45° . In this chamber rat can move freely but can not run away. This chamber allows an unhindered observation of the injected paw movement. The formalin-induced pain behaviors were graded according to the pre-determined criteria; 0 - normal weight bearing on the injected paw, 1 - little or no weight on the injected paw, 2 - elevation of the injected paw without any contact with floor surface, and 3 - licking or shaking the injected paw. Because licking or shaking activities were almost continuous for about the first 3min after formalin injection, pain behaviors could be graded without any difficulty. During the remaining period, a nociceptive score was assessed for each 2min observation period at a given time. When the rat kept the injected paw elevated or licked and shaked it for more than two-thirds of the observation time, pain score of 2 or 3 was given respectively.

Changes in the tail flick latency, hot plate latency and formalin-induced nociceptive score were assessed prior to and after administration of DPH and CBZ. DPH (20 and 40 mg/Kg) and CBZ (20 mg/Kg) were administered intraperitoneally 20 min before formalin injection. In 11 rats, CBZ was administered repeatedly for 6 days at the dose of 20 mg/Kg/day and the formalin-induced pain score was assessed at least 12 hours after the last administration. Also examined were the effects of strychnine and picrotoxin (1 mg/Kg, s.c.) on the CBZ-evoked inhibition of the formalin-induced pain behaviors.

RESULTS

Changes in the tail flick and the hot plate latencies following intraperitoneal administration of DPH(40 mg/Kg) and CBZ(20 mg/Kg)

To examine the analgesic effects of DPH and CBZ on the acute pain, the tail flick and hot plate latencies were assessed before and 30min after intraperitoneal administration of drugs and then summarized in Fig.1.

DPH showed a trend to reduce tail flick latency and to increase hot plate latency, but these changes were not statistically significant (Fig.1. A & B). As in the case of DPH, tail flick and hot plate latencies were not changed significantly even after intraperitoneal administration of CBZ. (Fig. 1. A & B).

DPH- and CBZ-induced changes in the tonic pain behaviors

Subcutaneous injection of formalin into the plantar surface of hind paw evoked a typical biphasic pain response. The early short-lasting response was caused immediately after injection of formalin, reached the peak level within about 2min and thereafter subsided gradually. About 10-15 min after formalin injection, the late sustained pain behaviors recurred and peaked at 30 to 40 min after formalin injection. Even 60min after formalin injection, this late tonic pain did not completely subside.

When DPH (20 and 40 mg/Kg) was administered intraperitoneally 20min prior to the formalin injection, high dose of DPH appeared to suppress the early phasic response more strongly compared to low dosage, but both low and high doses of DPH inhibited the late tonic pain behaviors to a similar extent (Fig. 2).

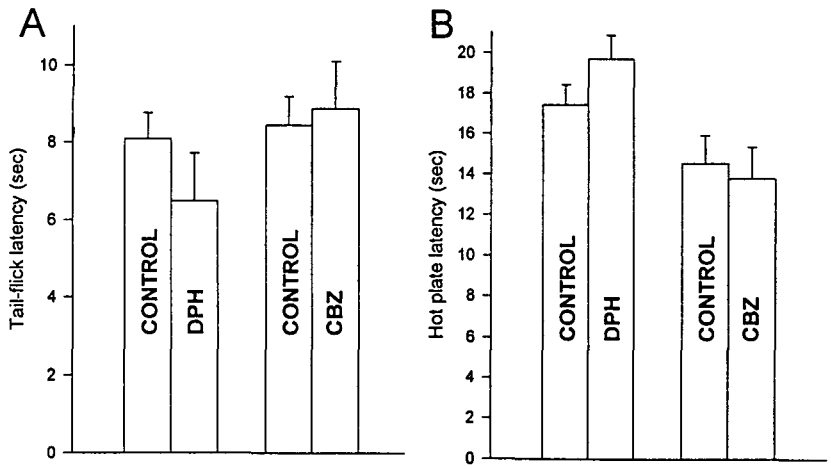

Fig. 1. Changes in tail-flick latency (A) and hot plate latency (B) before and after intraperitoneal administration of diphenylhydantoin(DPH, 40 mg/kg) and carbamazepine (CBZ, 20 mg/kg). Each value represents the mean ± S.E.

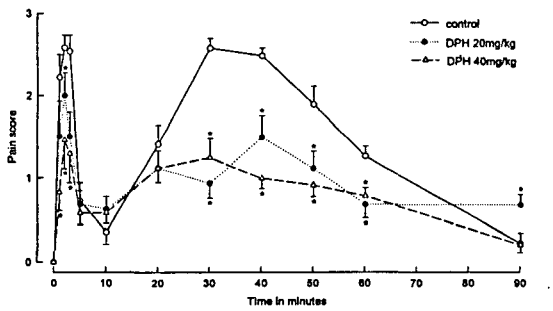

*Fig. 2. Changes in the formalin-induced pain behaviros following intraperitoneal administration of diphenylhydantoin (20 mg/kg and 40 mg/kg, DPH) in the rats. Each value represents the mean ± S.E. *; represents significant difference from the control group and P-values are less than 0.05. This principle can be applied to all other figures.*

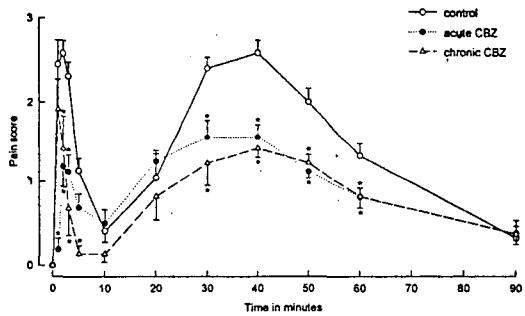

Fig. 3. Inhibitory action of acutely (20 mg/kg, i.p.) and chronically (20 mg/kg/day, i.p., for 6 days) administered carbamazepine (CBZ) on the formalin-induced pain behaviros in the rats. Each value represents the mean ± S.E.

Similarly, a single intraperitoneal administration of CBZ (20 mg/Kg) caused a strong inhibition of both early and late pain responses induced by formalin injection (Fig. 3). We could not find any great differences in the inhibitory action of single dose DPH and CBZ on the early phasic and late tonic components in formalin-induced pain behaviors. Repeated administration of CBZ for 6 days also produced a strong suppression of the late tonic pain responses to an extent similar to a single administration of CBZ but showed weaker inhibitory action on the early phasic pain responses compared to a single administration.

In order to examine the effects of strychnine and picrotoxin on the CBZ-induced inhibition, 1 mg/Kg of strychnine and picrotoxin was administered

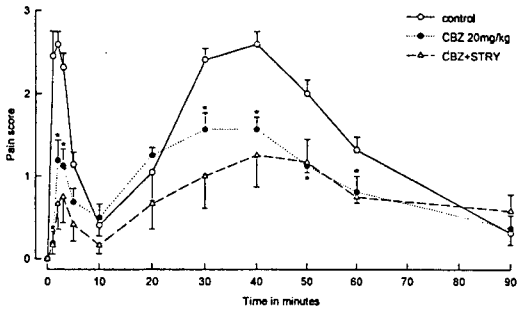

Fig. 4. Effects of strychnine (1 mg/kg, i.p.) on the inhibition of formalin-induced pain behaviros produced by intraperioneal administration of carbamazepine (20 mg/kg, CBZ). Each value represents the mean ±S.E.

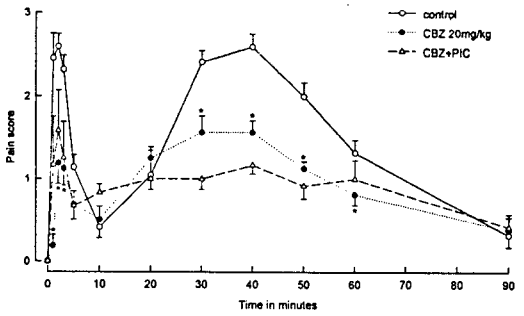

Fig. 5. Effects of picrotoxin (1 mg/kg, i.p.) on the inhibition of formalin-induced pain behaviros produced by intraperitoneal administration of carbamazepine (20 mg/kg, CBZ). Each value represents the mean ±S.E.

intraperitoneally 5min prior to formalin injection, respectively. As can be seen in Fig. 4 and 5, the CBZ-induced inhibition on the early phasic and the late tonic pain responses were not attenuated even after intraperitoneal administration of strychnine and picrotoxin.

DISCUSSION

A number of behavioral and electrophysiological methods have been developed in order to study nociception in animals. In most of these studies, the responses to a brief noxious stimulus such as radient heat or pinching the skin were recorded and analyzed. Though the employment of acute pain model is simple and convenient, the results obtained in this model are rather conflicted. While lesions made in the dorsolateral funiculus (DLF) or the nucleus raphe magnus (NRM) attenuated morphine analgesia in tail flick test, they did not attenuate or rather potentiated effect of morphine in the formalin test (Abbott & Melzack, 1982; Ryan et al, 1985). N-methyl-D- aspartate (NMDA) antagonists such as MK-801 and 2-amino-5-phosphonopentanoic acid (AP-5) have been known to produce a dose-dependent antinociception in the formalin-induced tonic pain (Coderre & Melzack, 1992; Yamamoto & Yaksh, 1992) but they did not have any inhibitory effect on the acute thermal nociception (Yaksh, 1989), the acute C fiber-evoked activity of spinal neurons (Dickenson & Sullivan, 1990) and the monosynaptic excitation (Davies & Watkins, 1983). All these experimental findings provide evidence that neural substrates to be involved in the acute and tonic pains are different.

In the present study, DPH and CBZ did not have any inhibitory effects on acute pain assessed by tail flick and hot plate tests. However, they significantly inhibited the formalin-induced tonic pain behaviors. The results of this experiment agree well with the contention that phasic and tonic pains may utilize the different pain suppression mechanisms (Abbott & Melzack, 1982; Ryan et al, 1985). From the present experiment, we do not know different neural pathways which are implicated in the phasic and tonic pains. The mechanism by which DPH and CBZ induce antinociceptive action is far from clear, but can be deduced from their known functions.

Formalin-injection induces biphasic behavioral pain responses for which C fibers are prerequisite (Dickenson & Sullivan, 1987) and causes the release of substance P (SP) especially during the early phase (Kuraishi et al, 1989) and excitatory amino acids during the late phase (Skilling et al, 1988; Coderre & Melzack, 1992). Intrathecal administration of SP antagonist attenuates formalin-induced phasic pain

without any effect on the late sustained pain behaviors (Ohkubo et al, 1990). In contrast, NMDA antagonists such as MK-801 and AP-5 more strongly suppress the late pain responses than the early phasic pain (Coderre & Melzack,1992; Yamamoto & Yaksh, 1992). Ohkubo et al(1990) reported that somatostatin antagonist and depletor selectively inhibited the late tonic pain responses with no effect on hot plate and tail pinch test. The early phasic pain results from direct stimulation of C fiber while the late tonic pain is supposed to be caused by acute inflammation. This dissociation of the phasic pain from the tonic pain is based primarily on the observation that anti-inflammatory agents reduce pain during the late phase but have no effect on the early phasic pain (Hunskaar & Hole, 1987; Malmberg & Yaksh, 1992). These experimental evidences indicate that inflammatory products such as histamine, serotonin, bradykinin and prostaglandin act together with released neurotransmitters (Shibata et al, 1989) and cause sustained pain behaviors resulting from the peripheral and central sensitization.

As mentioned in introduction, the anticonvulsants have 3 major properties to reduce sodium- and calcium-dependent action potential (Mclean & Macdonald, 1982; Willow et al, 1984), to facilitate the GABA-mediated inhibitory mechanism (Lee et al,1979; Macdonald & Bergey, 1979) and to inhibit EPSP (Selzer, 1978; Schneiderman & Schwartzkroin, 1982). Most of the inhibitory action of DPH and CBZ on noxious input has been known to be mediated in the spinal cord (Kim et al, 1993). Putting all these informations together, we can reach the conclusion that DPH- and CBZ-induced inhibitions of action potential and calcium influx suppress the presynaptic release of neurotransmitters responsible for nociception and then reduces signal transmission, especially pain signals, in the spinal and supraspinal levels.

Many workers reported that the anticonvulsants selectively augmented the GABA-mediated inhibition with no effect on the responses to glycine and glutamate (Ayala et al, 1977; Macdonald, 1978; Macdonald & Barker, 1979). In the present study, CBZ strongly inhibited the formalin-induced pain behaviors but strangely enough, the CBZ-induced inhibitory action was not changed even after intraperitoneal administration of picrotoxin and strychnine. This result indicates that the CBZ-induced antinociception is independent of GABA and glycine mechanisms.

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