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Cerebroventricular Injection of Cigarette Smoke Condensate Produce Generalized Seizures Decreased by Muscarinic Receptor Antagonist in Rats

Jawad Laadraoui and Abderrahman Chait

Abstract

Tobacco smoke is a complex multicomponent system, in which more than 4800 compounds have been identified by chromatographic techniques; many of these compounds are carcinogenic. However, there is a great deal of research into the association between smoking and diseases such as heart attacks, strokes and cancers. Nevertheless rare are the studies on the association between smoking and epilepsy because the exact roles of smoking and nicotine use in epilepsy have not been well examined. In this study the authors evaluate the convulsive effects of intracerebroventricular administration of cigarette smoke condensate in rats and compare intensity of seizures with kainic acid-induced seizures as a model of epilepsy. The role of cholinergic system was also evaluated using mAChRs antagonist in cigarette smoke condensate (CSC) induced seizures. Results indicate that central injection of cigarette condensate provides an epileptic behavior similar to that induced by kainic acid. However a pretreatment with atropine reduced seizures and all their parameters.

Keywords: seizures, epilepsy, cigarette smoke condensate, intracerebroventricular, kainic acid

1. Introduction

In the world, about 1% of people suffer from epilepsy [1]. Modern anticonvulsants can prevent and decrease the intensity of these convulsions. However, about 30% of people with epilepsy have uncontrollable seizures although of drugs availability. It is also known that the therapy ineffectiveness and chronic toxicity of antiepileptic drugs drawbacks the treatment procedure for nearly 20% of the patients [2].

Tobacco smoke is a complex multicomponent system, in which more than 4800 compounds, many of which are known carcinogens. As a result, chronic obstructive pulmonary disease, chronic bronchitis, cardiovascular disease, emphysema, stroke and many forms of cancer are directly related to smoking [3].

In March 2012, the Food and Drug Administration established a long list containing 93 harmful and potentially harmful components (HPHCs) and an abbreviated list containing 18 HPHCs in tobacco products and tobacco smoke (**Table 1**).

However, seizure control in the majority of epileptic patients is achieved primarily through the pharmacotherapeutic action of drugs targeting membrane ion channels or glutamatergic or gabaergic neurotransmission [4], which is dependent on a wide variety of modifications., glutamate and GABA [5]. For example, a weak activation of the GABAergic system induces epilepsy [6]. Generally, the risk of epilepsy should be higher in chronic tobacco smokers; this behavior is due to toxic components of tobacco smoke that can lead to seizure behavior in humans and animals [7, 8].

Many components in tobacco smoke are associated with seizures or epilepsy (**Table 2**) [9]. For example, nicotine, when overdosed, caused seizures in human subjects. Nicotine, a parasympathomimetic alkaloid in tobacco when overdosed, caused seizures in human [10]. The carbon monoxide causes seizures that can be focal or generalized and may even present as a status of epilepticus [11, 12]. Ammonia, hexane, lead, cresol, arsenic, toluene and acetone are other chemicals found in tobacco smoke that can trigger seizures in humans or animals [13].

HPHCs in cigarette smoke	HPHCs in smokeless tobacco	HPHCs in roll-your-own tobacco[a] and cigarette filler
Acetaldehyde	Acetaldehyde	Ammonia
Acrolein	Arsenic	Arsenic
Acrylonitrile	Benzo[a]pyrene	Cadmium
4-Aminobiphenyl	Cadmium	Nicotine (total)
1-Aminonaphthalene	Crotonaldehyde	NNK[*]
2-Aminonaphthalene	Formaldehyde	NNN[**]
Ammonia	Nicotine (total and free)	
Benzene	NNK[*]	
Benzo[a]pyrene	NNN[**]	
1,3-Butadiene		
Carbon monoxide		
Crotonaldehyde		
Formaldehyde		
Isoprene		
Nicotine (total)		
NNK[*]		
NNN[**]		
Toluene		

See Ref. [4]. [*]*4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone.*

[**]*N-Nitrosonornicotine.*
[a]*Roll-your-own tobacco is defined in section 900(15) of the FD&C Act to mean "any tobacco product which, because of its appearance, type, packaging, or labeling, is suitable for use and likely to be offered to, or purchased by, consumers as tobacco for making cigarettes." The term cigarette filler is not defined in the FD&C Act. For purposes of this draft guidance, we intend cigarette filler to mean the cut, ground, powdered, or leaf tobacco that is a component of a cigarette.*

Table 1.
Abbreviated list of harmful and potentially harmful constituents (HPHCs) in tobacco products.

Chemicals in tobacco smoke	Seizure-inducing	Potentiating seizures
Nicotine	Nicotine patch (H)[a]	
Carbon monoxide	(H)	
5% carbon dioxide		Low dose increased the severity of the electroshock-induced seizures (M)
Arsenic		
Ammonia	(H), (R)	
Hexane	(H)	
Lead acetate	High dose induced forelimb	Potentiated PTZ-induced convulsions (M)
Toluene	clonus (M)	Increased susceptibility to aminophylline-induced seizures (IP) (M)
Cresol	(H)	
Selenium		
Zinc		
Copper		
Nickel		
Acetone	High dose caused seizures (M)	

[a]H: human; M: mouse; R: rat.

Table 2.
Effects of chemicals in tobacco smoke on seizures or epilepsy.

Animals have been a useful tool for elucidating the association between tobacco smoking or nicotine use and seizures or epilepsy. Nicotine induced seizure models include cats, mice, and rats, and it was reported that animals received nicotine via injection develop seizures but not through the automatic smoking machine (ASM) [14, 15]. Other studies have shown that the activity of seizure-inducing chemicals such as pentylenetetrazole [16], kainic acid, pilocarpine, had been enhanced by prior pretreatment with nicotine [17].

In this study, we have investigated the convulsive effect of CSC as a crisis model compared to the intensity of the kainic acid model of epilepsy in rats. Thus examine the role of the cholinergic system in cigarette condensate seizures using a treatment with cholinergic muscarinic ligand.

2. Epileptic behavior induced by cerebral injection of cigarette smoke condensate

2.1 Methodology

The objective of the experiment was to demonstrate that a treatment with cigarette smoke condensate provides an epileptic behavior similar to this induced by a kainic acid, who reduced by a pretreatment with atropine.

The preparation of the cigarette smoke condensate was carried out by a cooling system consisting of a VP800 vacuum pump that generates and removes cigarette smoke to a tube and a balloon where the cigarette condensate is recovered [13]. Male

Sprague-Dawley rats (3–4 months old, 230–250 g weight) were used in this experiment, All animals were treated according to European decree, related to the ethical evaluation and authorization of projects using animals for experimental procedures, 1st February 2013, NOR: AGRG1238767A efforts were made to minimize the number and suffering of animals used. Before the experience all rats are anesthetized with an intraperitoneal injection of Hydrate chloral (400 mg/kg (6%)), before being placed in a Horsley-Clarke stereotactic frame. The head is secured with two bars that are inserted into the ear canal to the inner ears. The muzzle is well fixed by an oral part. After shaving the head, we made a median skin incision 1.5 cm long. The skin is spread laterally to clear the bone surface and highlight the coronal suture with the points, Bregma forward and Lambda back. The coordinates of the injection point with respect to the Bregma point are determined using the stereotaxic atlas of Paxinos and Watson [18]. The stereotaxic coordinates of the ventricle were as follows: the incisor bar −0.92 mm behind Bregma, ±1.5 mm laterally to the sagittal suture and 3.2 mm from the top of the skull. With a suitable milling machine, small openings are made in the cranial box for the placement (unilaterally) of the guide cannulas of approximately 23 gauge and the stainless steel anchor screws that will fix these cannulas. Stainless steel stylets (30 gauges) are placed in each guide cannula to prevent obstruction. These cannulas are then fixed by white dental cement. Then, with the help of pink dental cement, we put a crown in order to protect this preparation against any collision with the walls of the cage in which the animals were placed for 8 days to recover from surgery and eliminate anesthesia [13].

The animals were divided into four groups of six animals each. Control (saline 9%), kainic acid (1 µl/rat), CSC extract (2 µl/rat), atropine + CSC (Atr + CSC), a CSC was injected before 30 min of intraperitoneally injection of atropine (1 ml/kg).

For the KA and CSC infusion, the animals were gently retained by hand and the styles were removed from the guide cannulae and introduced 27 gauge injection needles. Injection was carried out using a Hamilton syringe (10 µl) which is connected to an injection cannula by a polyethylene catheter filled with distilled water. This was lowered into the guide cannula to a distance of 1.5 mm below its lower end to reach the target structure.

Total volume of 1 µl/rat for kainic acid and 2 µl/rat for CSC injected solutions were administered for a period of 60 s, and then the injection needles were left in the guide cannulae for a supplementary period of 60 s to facilitate drug delivery.

The animals were placed in the convulsion cage for 1 day before the beginning of the experiment to adapt the animals in the new environment.

A seizure was caused by intracerebroventricular injection of kainic acid and SCC. Immediately after the injection, each rat was placed in the center of the cage and its behavior was recorded and monitored within 90 min. The epileptic and mortality behaviors observed were classified as follows: latency of the seizure, latency of the tonic-clonic seizure, duration of the tonic-clonic seizure [13].

The scoring of seizures severity after KA injection was recorded during observation period (90 min) according to the scale:

0: normal activity; 1: immobility and/or staring; 2: rigidity, extension of the tail, swaying of the head; 3: repetitive movements, bilateral paws, breeding, tremor of hind limbs; 4: minor or flickering convulsions, jumps, falls; 5: tonic-clonic or multiple convulsions and/or appearance of score 4; 6: severe tonic-clonic seizure; 7: death [13].

The rats were randomly tested and to avoid the presence of odors that could lead to a change in behavior the convulsive cage was cleaned at the end of each test. Each rat has been subjected to a seizure test only once, and seizures have always occurred between 1:00 pm and 4:00 pm to minimize the confusional effects of the circadian rhythm [19].

2.2 Results

The results indicate that cigarette smoke condensate causes similar seizure behavior to this induced by kainic acid. However, the atropine-treated group showed a significant decrease in the convulsion score compared to the kainic acid group (**Figure 1**). There were no significant differences between the kainic acid and group treated with CSC concerning the time latency of seizures. However this parameter was significantly increased after treatment with atropine (**Figure 2**) [13].

Regarding to the latency of tonic-clonic seizures, the atropine-pretreated group showed a maximum value of score, which decreased significantly in the CSC group (**Figure 3**). However duration of tonic-clonic seizures was significantly decreased in the group pretreated with atropine compared to kainic acid and CSC groups. Nevertheless there is no significant deference between kainic acid and the CSC treated groups in the latency and duration of tonic-clonic seizures (**Figure 4**) [13].

2.3 Discussion

In this study, the authors explore the effects of intracerebroventricular pretreatment with tobacco smoke condensate on seizures and compare its severity to the administration of chemical-induced seizures, such as kainic acid as an important agent for the studying the function related to the excitatory transmission of amino acid-like neurotoxic powerful glutamate used in the rat [20]. The main finding of our study was that the central injections of CSC induce an epileptically behavior in rats. Furthermore, time latency of seizures; duration and latency of tonic-clonic seizures were significantly similar in the experimental groups. The modulation of the cholinergic system in the rat brain by activation or blockade of cholinergic receptors using atropine as a cholinergic antagonist has shown us the pathway and mechanism by which the preparation of SCC generates epileptic behavior. In this case, our results indicate that pretreatment with atropine reduced the intensity of seizures as well as other parameters recorded in the rat.

Nicotine is a major alkaloid in tobacco smoke, cigarette smoking has been the most famous method to intake nicotine [9]. Several laboratory studies were found that administration of low dose of nicotine produces behavioral effects
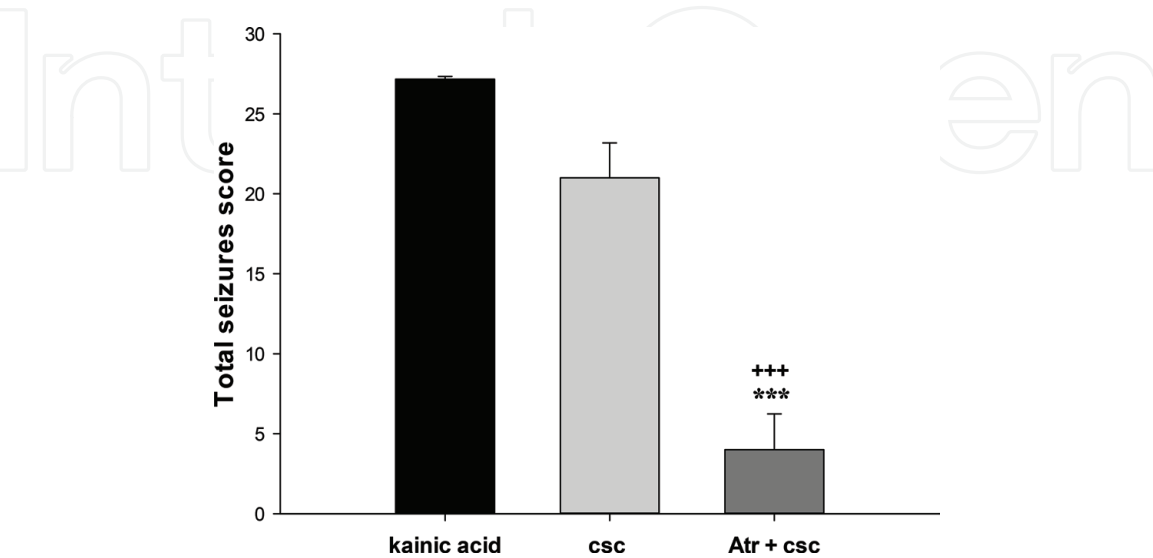

Figure 1.
Total seizure scores of all the groups. Rats received intracerebroventricularly administration of a cigarette smoke condensate (CSC), kainic acid and cigarette smoke condensate after intraperitoneally administration of atropine (atropine + CSC). Mean ± SEM: ***P < 0.001 significantly different from the kainic acid group. *\+\+\+P < 0.001 significantly different from CSC extract.*
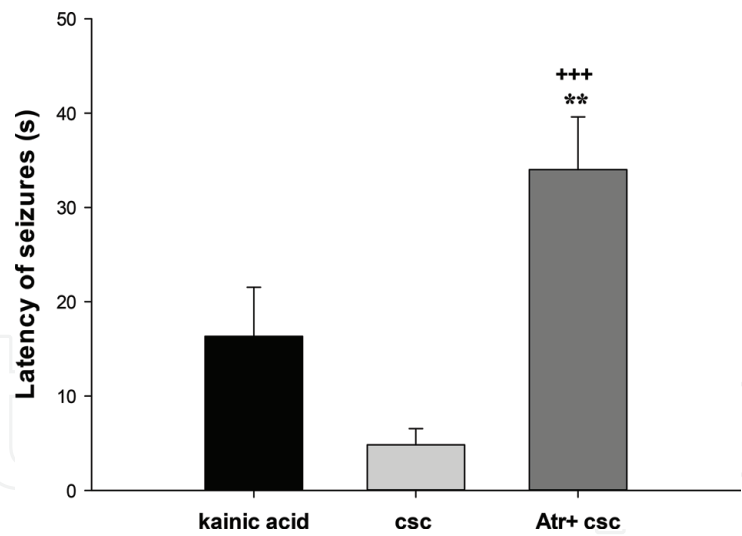
Figure 2.
Effect of intracerebroventricularly administration of kainic acid and cigarette smoke condensate (CSC) on latency of seizures. Mean ± SEM: **P < 0.01 significantly different from the kainic acid group. \+++P < 0.001 significantly different from CSC extract.
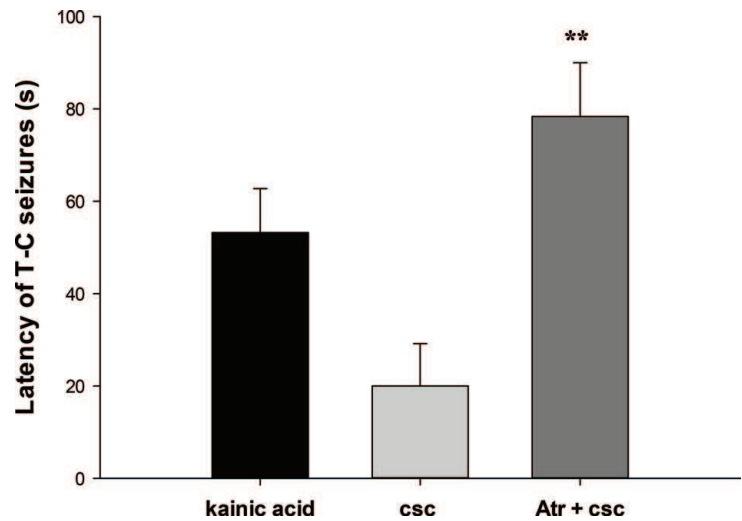

Figure 3.
Total duration of seizures after intracerebroventricularly administration of kainic acid and cigarette smoke condensate (CSC), in a 90 min total time of epileptic behaviors recording. Mean ± SEM. **P < 0.01 significantly different from CSC extract.

like nociception [21], locomotor activity [22], memory, learning, attention [23] and decrease or reduce anxiety [24], whereas high doses of nicotine cause seizures [25–27].

In our study, the central administration of CSC induces an epileptic behavior manifested by tonic-clonic seizures similar to this provoked by kainic acid. These results according to several electrophysiological studies indicated that intracerebroventricular administration of nicotine produces tonic-clonic seizures that have origin in the hippocampus structure [23, 25, 27].

Biochemical and pharmacological data have suggested that implicates the contribution of $\alpha 4\beta 2$ and $\alpha 7$-containing nAChRs situated in GABAergic interneuron, in the generation of nicotine induced seizures [25, 28]. Conti-Tronconi et al. [29] had proposed that $\alpha 7$-nAChR subtype to underlie nicotine induced seizures for the reason that seizures sensibility is significantly correlates with quantity of α-bungarotoxin binding sites exists in the hippocampus. Several studies have examined to define the interrelationship between the nicotinic cholinergic system
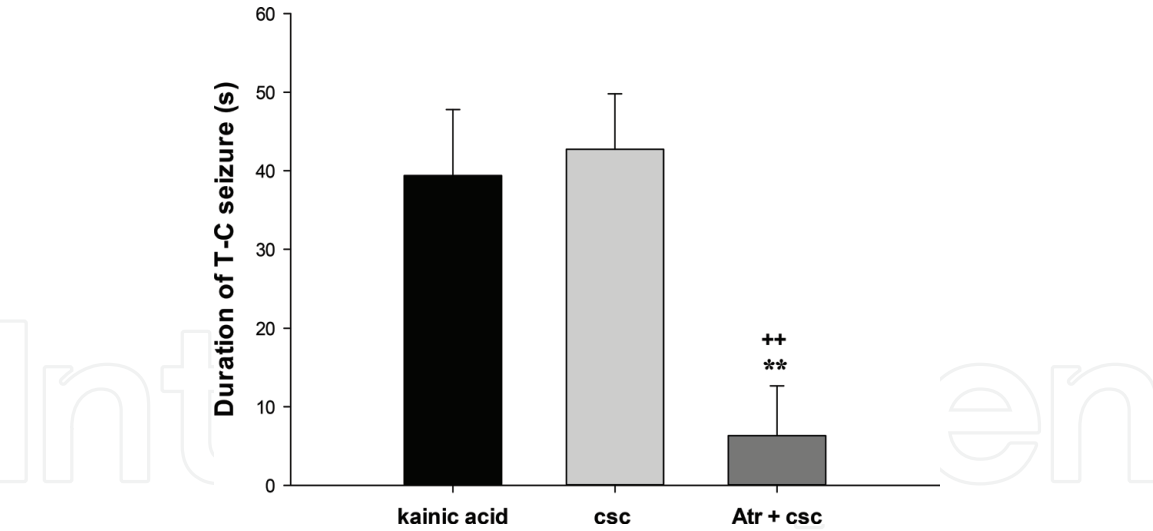
Figure 4.
Duration of tonic-clonic seizure after intracerebroventricularly administration of kainic acid and cigarette smoke condensate (CSC). Mean ± SEM: **P < 0.01 significantly different from the kainic acid group. ++P < 0.01 significantly different from CSC extract.

with the excitatory glutamatergic system and inhibitory gabaergic neurotransmitter in the brain. New study have reported that the α7-containing nAChRs receptors activation situated on glutamatergic nerve terminal conducing to synaptic liberation of glutamate which in turn stimulate *N*-methyl-D-Aspartate (NMDA) receptors located on pyramidal cells in of the hippocampus, are the most important mechanism that leads to nicotine seizures [25]. Though, other studies have demonstrated the contribution of α4β2 as well as α7-containing nAChRs receptors, localized in gabaergic interneurons in the creation of nicotine induced seizures [28, 30]. Contradictory to the glutamatergic hypothesis mentioned above, Dobelis et al. [28], proposed that nicotine-induced excitation was principally on relationship of pyramidal cells disinhibition in the hippocampus due to desensitization of α4β2 nAChR subtype situated on the cell bodies and dendritic terminals of pre-synaptic gabaergic interneurons.

Our results also indicate that pretreatment with atropine, a cholinergic blocking agent decreased score and latency of total seizures and tonic-clonic seizures of CSC-induced seizures; this experiment indicate that cholinergic circuit have an essential role in the mechanism underling the generation of seizures by cigarette smoke condensate. Supporting to this finding, numerous studies reporting that atropine acts as an anticonvulsant tool reducing the incidence and effectiveness of convulsions induced by an organophosphorus nerve agent [31]. A new study reporting that the anticonvulsant effects of atropine diminished with the seizures progression in a soman-induced seizures model in rats. This anticonvulsant action vanishes when the seizures had persisted for a period of 40 min [32]. Gholami et al. [33] reported a significant effect of cholinergic ligand in the pentylenetetrazole-induced epilepsy model in the rat hippocampus and they founded that cholinergic agonists leads to an augmentation of tonic-clonic seizures severity and rate mortality, however cholinergic antagonist decrease duration of tonic-clonic seizures, these data are in agreement with our study.

3. Conclusion

Data had revealed that the intracerebroventricular injection of CSC induces tonic and clonic seizures characterizing epieltical behavior similar to this triggered

by acid kainic model of seizures and a significant modulatory effect of cholinergic antagonist ligands in the CSC induced seizures. In this epilepsy model, CSC led to an increment of time latency, duration and latency of tonic-clonic seizures; while pretreatment with cholinergic antagonist increased all parameters recorded. This finding provide supplementary support for data that tobacco and specially nicotine had convulsing actions and confirmation of novel CSC induced seizures model of epilepsy in rats.

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