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Neuronal responses to cannabinoid receptor ligands in the solitary tract nucleus

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Abstract

Our previous study showed many neurons in the subpostremal division of the nucleus tractus solitarii to be cannabinoid-sensitive. In order to further investigate this sensitivity, single unit activity was recorded extracellularly in rat hindbrain slices, and the effects of bath application of Δ^9-tetrahydrocannabinol and of two synthetic cannabinoid receptor agonists were analysed and compared to each other. Approximately half the recorded neurons responded to agonists, and most of the neurons exposed to two of the agonists reacted similarly to both. The involvement of cannabinoid CB_1 receptors in neuronal sensitivity to Δ^9-tetrahydrocannabinol is supported by these data and by the effects of *N*-piperidin-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide, hydrochloride (SR 141716A), a compound which is considered to be a selective antagonist and/or a selective inverse agonist of this receptor type.

Keywords: Δ^9-Tetrahydrocannabinol; Nucleus tractus solitarii; Unit activity; Cannabinoid receptor

1. Introduction

Because of its hydrophobic nature, Δ^9-tetrahydrocannabinol, the main active component of marijuana, has long been assumed to exert its effects by disrupting the lipid bilayer of the cell membrane. Recently, evidence for the existence of cannabinoid receptors and of endogenous cannabinoid ligands led to the view that most psychological and physiological effects of cannabinoid molecules result from interaction with stereoselective receptors instead of with non-specific binding sites in the plasma membrane (Makriyannis, 1995). Two cannabinoid receptor subtypes have been cloned and sequenced. One (CB_1) is mainly distributed within the central nervous system (Matsuda et al., 1990, 1993), whereas the other (CB_2) is peripherally located and is expressed in particular in the immune system (Munro et al., 1993).

In our previous study carried out on rat hindbrain slices (Himmi et al., 1996), local application of Δ^9-tetrahydrocannabinol modified the firing rate of about 50% of the neurons recorded in the caudal division of the nucleus tractus solitarii, below the area postrema. This subnucleus, named the subpostremal nucleus tractus solitarii (Barraco et al., 1992), is the recipient of vagal afferent fibers originating in the digestive tract (Contreras et al., 1982), including those which convey emetogenic signals (Boissonade et al., 1994). It is a glycemia-sensitive structure (Yettefti et al., 1995, 1997) and may be involved, along with the area postrema, in the control of glucoprivic eating, i.e., in the facilitation of feeding behavior observed when a decrease in cellular glucose utilization is induced by intracerebroventricular infusion of an antiglycolytic glucose analog (Bird et al., 1983). Accordingly, our previous observations that the Δ^9-tetrahydrocannabinol-sensitive neurons respond to a change in glucose level and to a $5\text{-}HT_3$ receptor agonist were discussed in relation to the orexigenic and nausea-reducing effects of cannabinoids (Himmi et al., 1996).

The present study was undertaken to confirm the particular sensitivity to cannabinoids of the subpostremal nucleus tractus solitarii, and to examine its possible mediation by the cannabinoid receptors located in this area (Herkenham et al., 1991; Mailleux and Vanderhaeghen, 1992). To this end, neuronal responses to Δ^9-tetrahydrocannabinol were compared with responses to two potent analogues which are structurally different from each other. The first is the aminoalkylindole (*R*)-(+)-[(2,3-dihydro-

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5-methyl-3-[(4-morpholinyl)methyl]-pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl](1-naphtalenyl) methanone (WIN 55,212-2). The second is the non-classical cannabinoid [1α,-2ß(*R*)5α]-(−)-5-(1,1-dimethyl-heptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)-cyclohexyl] phenol (CP-55,940) (Martin et al., 1995). Any response to Δ^9-tetrahydrocannabinol mediated by stereoselective receptors should be mimicked by administration of these compounds.

Whereas the agonists, CP-55,940 and WIN 55,212-2, bind to both subtypes of human cannabinoid receptors (Felder et al., 1995), *N*-piperidin-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide, hydrochloride (SR 141716A) is a selective ligand of the cannabinoid CB_1 receptor. Previously considered as a pure antagonist (Rinaldi-Carmona et al., 1994), it can also function as an inverse agonist (Bouaboula et al., 1997). In order to find support for the hypothesized involvement of cannabinoid CB_1 receptors in cannabinoid sensitivity in the nucleus tractus solitarii, we tested the possibility of either a proper response to SR 141716A opposite to the response to agonists, or a blocking effect of SR 141716A on the agonist-induced responses.

2. Materials and methods

Male Wistar rats weighing 200–300 g were anesthetized by halothane inhalation (Fluothan, Coopers) and killed by decapitation. The brain was quickly removed and placed in chilled artificial cerebrospinal fluid saturated with 95% O_2 and 5% CO_2, and containing 127.5 mM of NaCl, 5 of KCl, 1.25 of K H_2 PO_4, 2 of Mg SO_4, 3 of Ca Cl_2, 25.5 of $NaHCO_3$, and 5 of glucose. The medulla oblongata was microdissected using previously described techniques (Dekin et al., 1987). Then, 300-μm thick coronal slices were cut at the area postrema level with an oscillating tissue slicer (Campden Instruments) and transferred into a preincubation chamber filled with oxygenated artificial cerebrospinal fluid at room temperature.

After more than 2 h of preincubation, the recording session started in a submerged type of recording chamber (0.5 ml) perfused at a rate of 1.5 ml/min with oxygenated artificial cerebrospinal fluid containing 4 mM glucose. The temperature of the chamber was kept at 34°C. The bottom of the chamber was coated with Sylgard (Dow Corning) so that slices could be fixed with platinum staples. The perfusion medium entered the chamber close to the slice.

Bath application of drugs was performed by injection into the perfusion inlet tubing. Four drugs were used in the study. WIN 55,212-2 (Research Biomedicals International), CP-55,940 (generously donated by Pfizer Pharmaceuticals, Groton, CT, USA), and SR 141716 A (generously donated by Sanofi Recherche, Montpellier, France) were dissolved in dimethylsulfoxide at 1.9 10^{-3} M, 2.6 10^{-3} M and 0.9 10^{-3} M, respectively, and the final concentrations in the recording chamber were 0.25–1 μM, 0.5–1 μM and 0.5–1 μM, respectively. Δ^9-tetrahydrocannabinol (Sigma) was dispersed in artificial cerebrospinal fluid using polyvinylpyrrolidone as a carrier, according to a standard procedure (Fenimore and Loy, 1971) (final concentration in the chamber: 6.4 μM). Control applications of dimethylsulfoxide and polyvinylpyrrolidone were routinely carried out.

Single unit activity was recorded extracellularly with a glass microelectrode (tip diameter: 2–3 μm) filled with 3 M NaCl. Electrodes were placed in the subpostremal nucleus tractus solitarii under microscope observation, according to atlas plates (Barraco et al., 1992). Amplified spikes were observed on a storage oscilloscope and selected by a window discriminator. The number of spikes per 5 or 10 s was counted by the timer of an interface adapter board (National Instruments) inserted into a computer (Macintosh, Apple) and the time course of the firing frequency was plotted on the screen. Once the spontaneous activity had become stable, drugs were injected. When the unit activity changed within less than 5 min after the drug application, the mean discharge frequency measured during the period of altered activity was compared to that measured for a control period of 5 min immediately preceding the stimulus. Student's t-test was used to check that the response was statistically significant.

3. Results

A total of 61 neurons were investigated in the study.

3.1. Responses to synthetic agonists of cannabinoid receptors

Possible effects of bath applications of WIN 55,212-2 were examined for 58 neurons. Twenty-seven (46.5%) responded after a latency of 98.7 ± 13.5 s (S.E.): 19 were activated (Fig. 1) and 8 had a decreased firing rate (Figs. 2 and 3).

Nineteen neurons were tested by bath application of CP-55,940. Ten (52.6%) responded after 97.8 ± 22.7 s: 6 were activated and 4 had a decreased firing rate (Fig. 2).

Of 16 neurons tested with both agonists, 14 reacted similarly. While 7 failed to be affected by WIN 55,212-2 and CP-55,940, 4 had an increased and 3 a decreased firing rate in response to both compounds (Fig. 2). Of the remaining 2 neurons, 1 responded to WIN 55,212-2 only, and the other to CP-55,940 only.

3.2. Response of the same neurons to Δ^9-tetrahydrocannabinol and to a synthetic agonist

Of the 16 neurons tested with bath applications of Δ^9-tetrahydrocannabinol and WIN 55,212-2, 4 responded to Δ^9-tetrahydrocannabinol only, while 12 reacted similarly to both compounds: 7 were unaffected, 3 were activated (Fig. 1) and 2 were depressed by both.

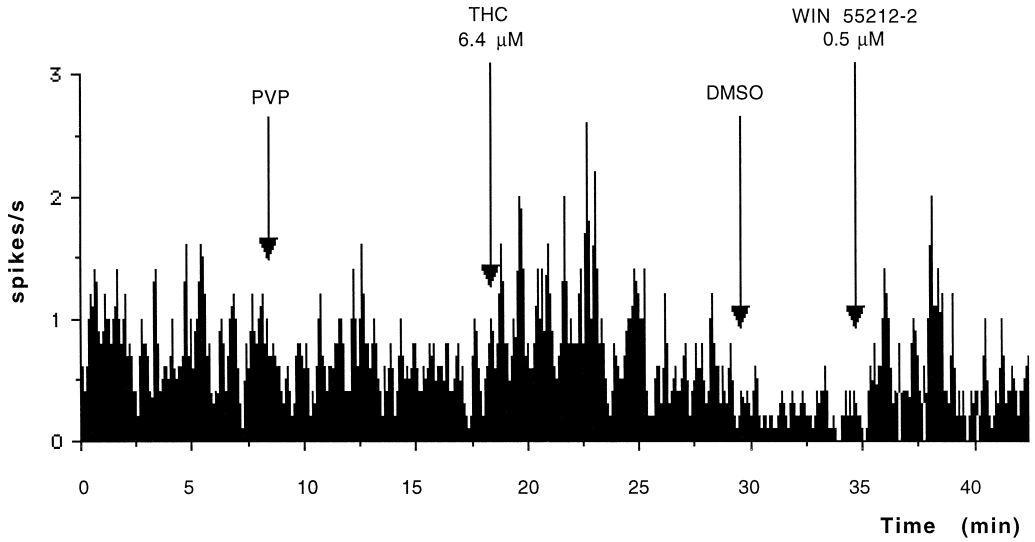

Fig. 1. Firing frequency of a neuron activated after bath application of either Δ^9-tetrahydrocannabinol (THC) or cannabinoid receptor agonist, WIN 55,212-2. The response failed to be reproduced by control application of either the Δ^9-tetrahydrocannabinol vehicle, polyvinylpyrrolidone (PVP), or the WIN 55,212-2 vehicle, dimethylsulfoxide (DMSO).

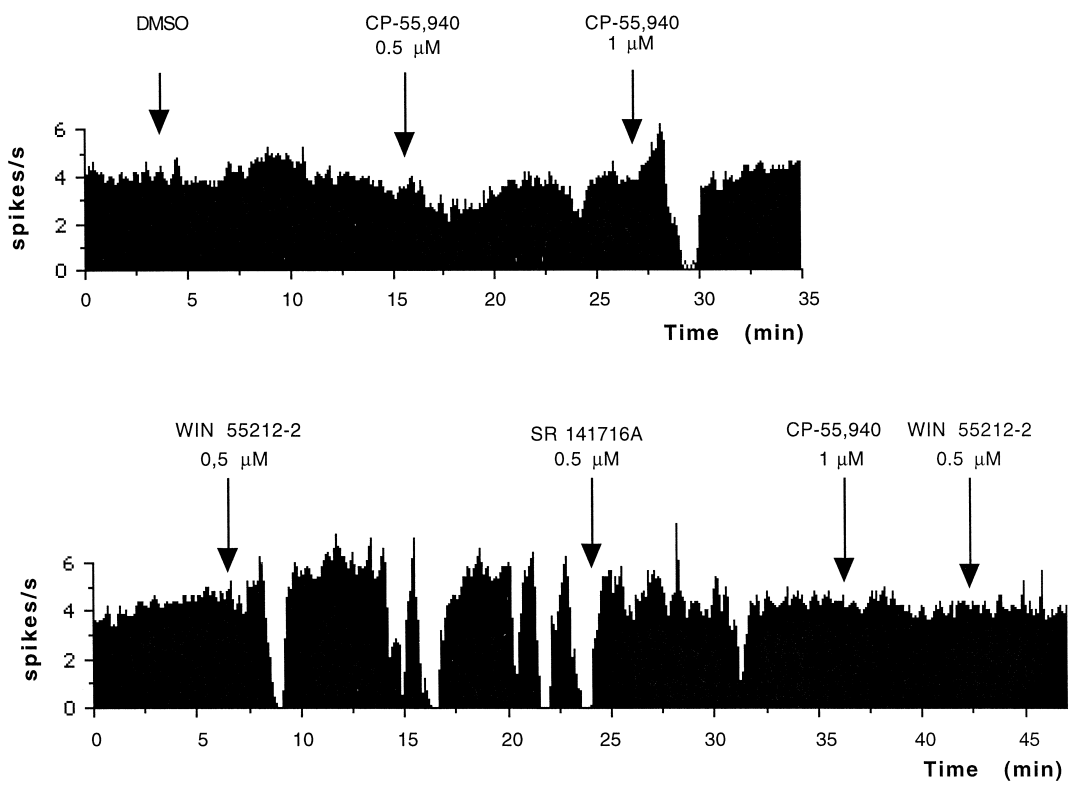

Fig. 2. Suppressive effects of the cannabinoid antagonist/inverse agonist, SR 141716A, on a neuron's responses to cannabinoid receptor agonists, WIN 55,212-2 and CP-55,940. Activity was unaffected by CP-55,940 0.5 μM and strongly depressed 2.5 min after application of CP-55,940 1 μM or WIN 55,212-2 0.5 μM, this depression being preceded by a brief excitatory episode. The application of WIN 55,212-2 0.5 μM was also followed by late episodes of depressed activity that were interrupted after the application of SR 141716A. These delayed episodes of decreased firing rate might represent spontaneous fluctuations rather than specific cannabinoid responses, as no such changes were evoked by CP-55,940 1 μM application. The early biphasic response to CP-55,940 and to WIN 55,212-2 (transient excitation followed by a strong depression) was not altered between the CP-55,940 1 μM and the WIN 55,212-2 0.5 μM applications, showing no sign of tolerance. The complete suppression of the early responses to CP-55,940 1 μM and WIN 55,212-2 0.5 μM after application of SR 141716A most likely results from the action of this antagonist/reverse agonist. Control application of the vehicle, dimethylsulfoxide (DMSO), had no effect.

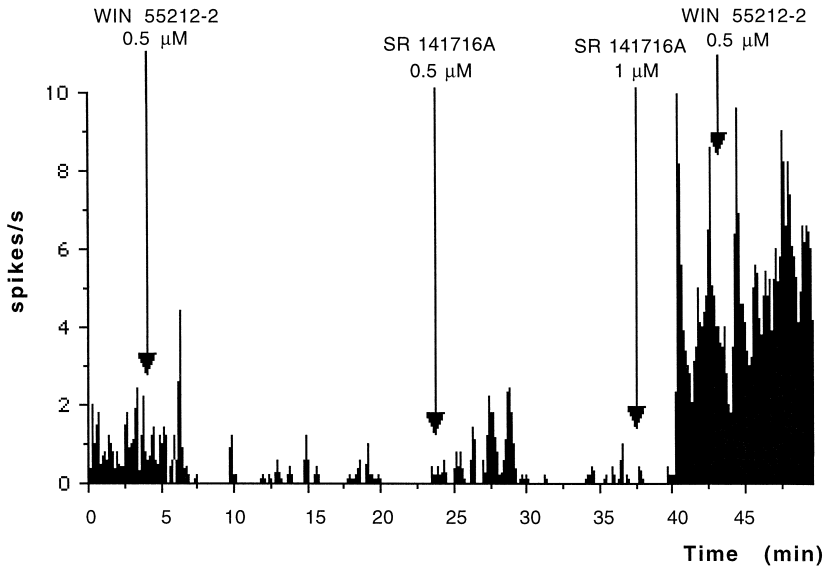

Fig. 3. Firing frequency of a neuron for which the involvement of CB_1 receptors in cannabinoid sensitivity was deduced from the observation that it responded in opposite directions to the cannabinoid receptor agonist, WIN 55,212-2 (decreased activity), and to the cannabinoid receptor antagonist/inverse agonist, SR 141716A (activation). When applied during the decreased firing frequency induced by WIN 55,212-2 0.5 μM, SR 141716A 0.5 μM only transiently restored basal activity. Strong sustained activation was evoked by SR 141716A 1 μM. No significant effect of WIN 55,212-2 0.5 μM was seen during this activation response.

Of 26 neurons responding to WIN 55,212-2, 4 and tested with a second cannabinoid (either CP-55,940, or Δ^9-tetrahydrocannabinol), 21 responded similarly to both compounds, ruling out, in most cases, the rapid occurrence of tolerance in this system.

3.3. Neuronal responses to SR 141716A

Seventeen neurons were tested with the antagonist/inverse agonist SR 141716A and either WIN 55,212-2 or CP-55,940. Of 9 neurons unaffected by the agonist, 8 also failed to respond to SR 141716A, and the other was depressed by it. Of the 8 neurons that responded to the agonist, 5 responded to SR 141716A, and the responses to the two compounds were in opposite directions (Fig. 3). The other 3 neurons did not respond to SR 141716A, but after the application of SR 141716A, a response to the agonist was no longer observed (Fig. 2).

4. Discussion

Together with our previous results (Himmi et al., 1996), the present data show that about half the neurons recorded in vitro in the subpostremal nucleus tractus solitarii modified their firing rate in response to bath applications of either Δ^9-tetrahydrocannabinol or synthetic analogs. The sensitivity of this area to cannabinoid is in keeping with radioautographic mapping studies of brain cannabinoid receptors, using tritiated CP-55,940. Mapping revealed that one of the highest concentrations of lower-brainstem binding sites is found in the caudal nucleus tractus solitarii (Herkenham et al., 1991; Mailleux and Vanderhaeghen, 1992).

The hypothesis that the response of nucleus tractus solitarii neurons to Δ^9-tetrahydrocannabinol is mediated by cannabinoid receptors, instead of by lipid bilayer-based mechanisms only, is compatible with two findings presented here. First, a majority of the neurons tested reacted similarly to the three different classes of cannabinoid molecules: classical cannabinoid (Δ^9-tetrahydrocannabinol), aminoalkylindole (WIN 55,212-2), and nonclassical cannabinoid (CP-55,940). Secondly, the cannabinoid receptor antagonist/inverse agonist, SR 141716A, induced a response in the direction opposite to that of the agonist response and/or blocked the response to the agonist.

However, Δ^9-tetrahydrocannabinol, as a highly lipophilic molecule, also directly affects the organization of membrane lipids, and results of biophysical studies suggest that the cannabinoid-receptor interaction may be influenced by perturbations of the lipids in the plasma membrane (Makriyannis, 1995). The bilayer-perturbating property of Δ^9-tetrahydrocannabinol is shared by other agonists such as WIN 55,212-2 and CP-55,940. A reduction in the ordering of the membrane lipids is caused by Δ^9-tetrahydrocannabinol, and even more by WIN 55,212-2 and CP-55,940, and leads to an increase in bilayer fluidity that might facilitate access of cannabinoid molecules to receptor sites by diffusion through the lipid medium (Bloom et al., 1997). The increase in membrane fluidity caused by the cannabinoids, Δ^9-tetrahydrocannabinol, WIN 55,212-2 and CP-55,940, however, does not correlate with their pharmacological efficacy (Bloom et al., 1997). Moreover, eliminating the influence of bilayer environment by solubi-

lization of brain cannabinoid receptors was found not to significantly affect receptor occupation or receptor coupling to the effector system (Houston and Howlett, 1993). It is also worth noting that the ranking of the three cannabinoids as membrane perturbers does not account for the difference in response latencies observed in our studies: lower values were obtained for Δ^9-tetrahydrocannabinol (Himmi et al., 1996) than for both synthetic analogs (present data). The opposite could be deduced from the facilitatory effect of these compounds on membrane fluidity, which should accelerate access to receptor sites for WIN 55,212-2 and CP-55,940 more than for Δ^9-tetrahydrocannabinol. The latency differences might result instead from the use of different vehicles (polyvinylpyrrolidone vs. dimethylsulfoxide).

The receptors that mediate nucleus tractus solitarii responses to cannabinoids most likely belong to the CB_1 subtype, since no cannabinoid CB_2 receptor has been described to date in the central nervous system (Pertwee, 1997). This view is supported by our observation that the CB_1-selective antagonist/reverse agonist, SR 141716A, blocked the response to the agonist or induced a response in the opposite direction.

The relevant receptors might be located on intrinsic neurons or on presynaptic terminals. The first possibility is supported by the in situ hybridization finding of slight to moderate cannabinoid receptor messenger RNA levels in the neuronal perikarya that compose the nucleus tractus solitarii (Mailleux and Vanderhaeghen, 1992). The second hypothesis, which assumes that the relevant receptors are located presynaptically, may account for the coexistence of nucleus tractus solitarii neurons responding to bath application of cannabinoid, some by increasing their firing rate, others by decreasing it. In particular, we discussed earlier the idea that such receptors are located on the central endings of vagal afferent neurons within the caudal nucleus tractus solitarii (Himmi et al., 1996). The possibility is supported by the cannabinoid-induced responses in the somata of the same neurons in the nodose ganglion (Fan, 1995). This hypothesis does not imply that the receptors belong to the CB_2 subgroup. These peripheral neurons might express cannabinoid CB_1 receptors as well: a number innervate the digestive tract, where the presence of cannabinoid CB_1 receptors has recently been demonstrated in the guinea pig (Pertwee et al., 1996). The cannabinoid CB_1 receptor is a G protein-coupled receptor often located on presynaptic nerve terminals, where it is expected to depress neurotransmitter release through inhibition of Ca^{2+} currents and activation of K^+ currents (Pertwee, 1997). Depending on whether the synapse is excitatory or inhibitory, this reduction in neurotransmitter release should result in postsynaptic depression or activation, respectively, accounting for the coexistence of the two types of responses recorded in the nucleus tractus solitarii. Sometimes, as in Fig. 2, the response to cannabinoids consists of a depression preceded by a brief activation. A possible interpretation of this dual effect is that the recorded neuron is under the tonic influence of inhibitory and activating inputs, both of which are depressed by cannabinoids: in presynaptic terminals, cannabinoids may (1) briefly block the release of an inhibitory transmitter through the induction of a transient A-type outward potassium current, similar to that induced by cannabinoids in hippocampal cells (Deadwyler et al., 1993), causing postsynaptic activation, and (2) block the release of an excitatory transmitter for a longer time, for example by inhibiting calcium conductance and causing postsynaptic depression.

The CB_1-mediated modulation of neurotransmission has been directly demonstrated in the hippocampus, where the evoked release of acetylcholine (Gifford and Ashby, 1996) and epinephrine (Schlicker et al., 1997) was found to be reduced by WIN 55,212-2 and increased by SR 141716A. Likewise, putative presynaptic cannabinoid CB_1 receptors located on vagal sensory terminals in the nucleus tractus solitarii might depress synaptic transmission of sensory visceral signals such as the nausea-inducing signals originating in the gut. It may be that the nausea-reducing effects of cannabinoids are partly mediated by such a mechanism.

In some neurons, SR 141716A triggered responses in the opposite direction to those evoked by agonists. It has been suggested, considering other intrinsic effects of SR 141716A, that the compound behaves as an inverse agonist rather than as a pure antagonist (Pertwee, 1997), in line with the concept proposed by Milligan et al. (1995). Thus, like other G protein-coupled receptors, the cannabinoid CB_1 receptor may fluctuate between two conformations: an inactive state and an active state. The effect produced by SR 141716A in the absence of other drugs would be due to its higher affinity for the inactive conformation than for the active one, resulting in a reduction of the basal activity of the effector cascade. This possibility has been confirmed in work on Chinese hamster ovary cells transfected with human cannabinoid CB_1 receptors, in which high constitutive mitogen-activated protein kinase and adenylcyclase activities were observed and were blocked by SR 141716A (Bouaboula et al., 1997). As mentioned by the authors, these data do not rule out the possibility that SR 141716A may have antagonistic properties. Therefore, intrinsic responses to SR 141716A may also be due to the interruption of the tonic activity of cannabinoid CB_1 receptors induced by an endogenous cannabinoid such as anandamide (Devane et al., 1992). In this case, the neuronal responses to exogenous cannabinoids in the subpostremal nucleus tractus solitarii may be physiologically relevant, insofar as they reflect the modulatory effect of endogenous cannabinoids on neuronal signaling in this area.

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