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European Journal of Pharmacology 337 (1997) 209–212

ejp

The triggering of spreading depression in the chick retina by nicotinic receptor agonists

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Received 12 June 1997; revised 28 July 1997; accepted 22 August 1997

Abstract

Spreading depression was evoked in vitro in retinas of 3–6 day old chickens by the nicotinic cholinergic agonists nicotine and cytisine. The response was reproducible and inhibited by the nicotinic cholinergic receptor antagonist mecamylamine and by the NMDA receptor antagonist -3-(2 carboxypiperazine-4-yl)-propyl-1-phosphonic acid (CPP). The response to nicotinic agonists was not inhibited by α-bungarotoxin. The data show that spreading depression can be evoked in the chick retina by α-bungarotoxin insensitive nicotinic acetylcholine receptor subtypes and that the response is dependent upon NMDA receptor activation. This nicotine evoked spreading depression was inhibited by cadmium chloride indicating the involvement of voltage sensitive calcium channels. It is therefore argued that nicotine evokes spreading depression indirectly, as a result of calcium sensitive glutamate release. The glutamate released thus exerting it's effects via NMDA receptors. © 1997 Elsevier Science B.V.

Keywords: Spreading depression; Nicotinic acetylcholine receptor; NMDA receptor; Glutamate

1. Introduction

Leao's spreading depression of electroencephalographic (EEG) activity is manifested by a slow negative potential wave which spreads across the cerebral cortex at a rate of 2–3 mm/min (Leao, 1944). Martins-Ferreira and Oliveira-Castro (1966) observed spreading depression in the chicken retina where light scattering changes make the phenomenon easily visible to the unaided eye.

It was postulated by Van Harreveld (1976) and Van Harreveld and Fifkova (1970, 1971) that the excitatory amino acid glutamate played a role in the chain of events triggering spreading depression. Subsequently Gorelova et al. (1987) in the rat cerebral cortex and Sheardown (1993) in the chicken retina have shown that the triggering of spreading depression requires activation of the NMDA subtype of the glutamate receptor family. Agonists selective for other glutamate receptor subtypes can trigger spreading depression in the chicken retina (e.g., AMPA, quisqualate, and kainate) but their effects can always be inhibited by selective NMDA receptor antagonists (Sheardown, 1993). This suggests that they exert these effects indirectly by releasing glutamate which thus evokes spreading depression via an action at NMDA receptors.

Acetylcholine has for a long time been known to be a neurotransmitter in the vertebrate retina (for references see Neal, 1976). Pourcho (1979) using cytohistochemical techniques showed that nicotinic cholinergic receptors were located in the mammalian retina at amacrine–amacrine and amacrine–bipolar synapses and several studies have shown the presence of nicotinic cholinergic receptors in the chick retina (e.g., Morgan and Mundy, 1982; Keyser et al., 1988). Furthermore Ikeda and Sheardown (1982) demonstrated that excitatory transmission in transient retinal ganglion cells in the cat was mediated by nicotinic cholinergic receptors. It has also been demonstrated that nicotine can enhance excitatory synaptic transmission in the CNS (McGehee et al., 1995) and evoke excitatory amino acid release in the rat spinal cord (Khan et al., 1996). The aim of this study therefore was to investigate if agonists at nicotinic cholinergic receptors were able to evoke spreading depression in the chick retina and if so, was the effect mediated indirectly via NMDA receptors through release of glutamate?

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2. Materials and methods

2.1. Spreading depression assay

Chickens (3–6 days old) were decapitated, the eyes enucleated and sectioned along the equatorial plane. After removal of the anterior chamber and the vitreous body, the posterior chamber of each eye was placed in a small petri dish containing a physiological saline solution (PSS) of the following composition (mM) NaCl (100), KCl (6.0), CaCl (1.0), $MgSO_4$ (1.0), $NaHCO_3$ (30), NaH_2PO_4(1.0), glucose (20). The solution was saturated with 100% oxygen and maintained at 26°C (see Sheardown, 1993).

The eyes were initially incubated in normal PSS for 30 min then carefully transferred to PSS containing various concentrations of nicotine or cytisine. Once in the solution containing a nicotinic agonist the latency for a spreading depression to start in each eye was measured. The presence of a white area 0.5 mm in diameter was taken as onset of spreading depression. A cut off time of 1 min was used. Following incubation in the agonist solution the eye cups were returned to normal PSS In antagonist studies the eye cups were incubated for 30 min in PSS containing various concentrations of either mecamylamine, α-Bungarotoxin or ($\pm$)-3-(2 carboxypiperazine-4-yl)-propyl-1-phosphonic acid (CPP), before being placed in PSS containing the antagonist plus the agonist to enable the latency to the triggering of spreading depression to be measured. The comparative potency of the agonists was quantified as the minimal effective concentration, that is the concentration (μM) capable of evoking a latency of at least 20 s in all eye cups tested (MEC). In the antagonist studies an increase in latency of 60 s more than the control time was considered to be 100% inhibition of spreading depression. The antagonist effects are therefore expressed as the percentage maximum inhibition obtained for a given concentration of the compound. The potency of an antagonist is quoted as the concentration of compound giving a 50% inhibition of spreading depression (EC_{50}). The MEC and EC_{50} values quoted are means of at least 6 determinations $\pm$ the standard error of the mean.

2.2. Drugs

Nicotine tartrate and cytisine were obtained from Sigma (St. Louis, MO, USA). CPP and α-bungarotoxin were obtained from Research Biochemicals International (Natick, MA, USA).

3. Results

Nicotine (MEC 30 μM $\pm$ 3.5) and cytisine (MEC 0.19 μM $\pm$ 0.04) both evoked spreading depression. The latency to the start of spreading depression was concentration dependent (see Fig. 1). The phenomenon appeared to be identical to that reported by Sheardown (1993) in that a milky white area spread slowly over the surface of the retina at a rate of 3–5 mm/min, usually starting at the edge of the tissue. These changes spread over the whole retina and were easily visible to the unaided eye.

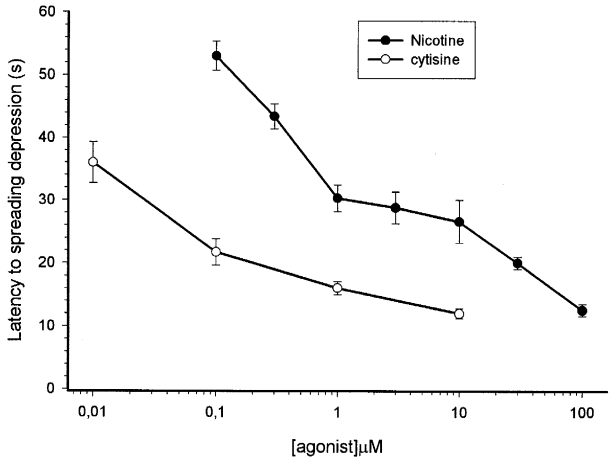

Fig. 1. The concentration-dependent triggering of spreading depression in the chick retina by nicotine and cytisine. Abscissa, log concentration of agonist; ordinate, latency to start of spreading depression. Each point on the graph is the mean of six determinations $\pm$ standard deviation.

To investigate the reproducibility of the response, in order to facilitate antagonist studies the eyes were exposed to 30 μM nicotine every 30 min for 2.5 h. The response latency was stable over that time period within a range between 19.7 and 21.8 s and there was no desensitization. Therefore a 30 μM concentration of nicotine was used for eliciting control responses in antagonist studies. Mecamylamine antagonized the response to nicotine in a concentration dependent manner (Fig. 2), the EC_{50} for mecamylamine was 1.5 μM $\pm$ 0.32. The NMDA receptor antago-

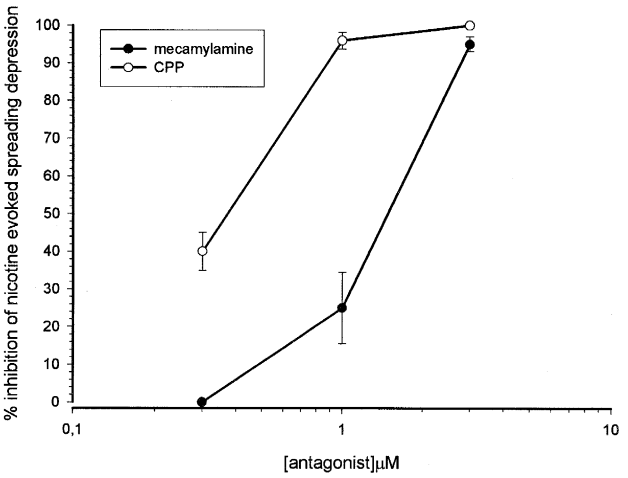

Fig. 2. Mecamylamine and CPP antagonize spreading depression evoked by 30 μM nicotine in a concentration dependent manner. Abscissa, log concentration of antagonist (mecamylamine or CPP), ordinate, % inhibition of spreading depression. Each point on the graph is the mean of six determinations $\pm$ standard deviation.

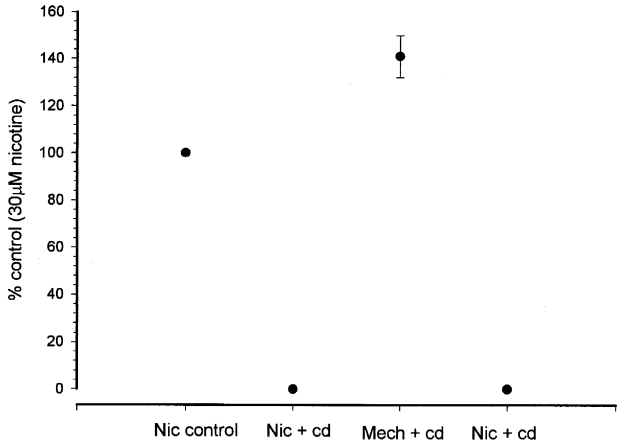

Fig. 3. The response to 30 μM nicotine in the presence of 100 μM cadmium chloride. Abscissa, treatment (nic = nicotine, cd = cadmium chloride, mech = mechanical stimulation with 28 g needle) time between determinations was 30 min. Ordinate, % of control latency to 30 mM nicotine. Each point is the mean of six determinations ± standard deviation.

nist CPP also inhibited the response to nicotine in a concentration dependent manner (EC_{50} 0.37 ± 0.04; Fig. 2). Nicotine evoked spreading depression was not inhibited by α-bungarotoxin at concentrations of up to 3 μM. Addition of 100 μM cadmium chloride to the PSS however, inhibited the triggering of spreading depression by nicotine (Fig. 3). Whilst the eye cups were incubated with cadmium the spreading depression was successfully evoked with a stab using a fine (28 g) needle, exposure to nicotine (30 μM) 30 min prior to and following the needle stab, in the presence of cadmium; failed to evoke spreading depression. This indicates that the prolonged exposure to cadmium had not simply poisoned the tissue and made it unresponsive.

4. Discussion

The data presented show that spreading depression can be reproducibly evoked in the chicken retina by the nicotinic acetylcholine receptor agonists nicotine and cytisine. The nature and appearance of the spreading depression responses were similar to those observed by Sheardown (1993). Acetylcholine is known to be a neurotransmitter in the vertebrate retina and acetylcholine and acetylcholinesterase inhibitors have long been known to have profound effects on retinal neurones (Straschill, 1968; Ames and Pollen, 1969; Masland, 1980). Many studies have demonstrated the presence of nicotinic acetylcholine receptors in the chicken retina. Morgan and Mundy (1982) demonstrated that nicotinic acetylcholine receptors were present on ganglion cell dendrites which made contact with cholinergic amacrine cells whilst Keyser et al. (1988) showed that nicotinic acetylcholine receptors were present in the inner nuclear and inner plexiform layers of the chicken retina, mainly on amacrine and ganglion cells. Tomita and Shimoda (1983) demonstrated that in the retina spreading depression seemed to be mediated by the ganglion cell layer.

The spreading depression responses produced by nicotine and cytisine were inhibited in a concentration dependent manner by low concentrations of mecamylamine suggesting that they were evoked by neuronal nicotinic acetylcholine receptors. However, the responses were not blocked by α-bungarotoxin at concentrations up to 3 μM thus they were evoked by an α-bungarotoxin insensitive subtype of nicotinic acetylcholine receptor. Several studies have shown that the chicken retina contains both α-bungarotoxin sensitive and insensitive nicotinic acetylcholine receptor subtypes. Whiting et al. (1991) demonstrated the presence of relatively high levels of α-bungarotoxin insensitive α3, β2-containing subtypes in the chicken retina whilst Britto et al. (1992) in chick retina described the presence of bungarotoxin sensitive and insensitive nicotinic acetylcholine receptor subtypes; with the inner nuclear layer containing the majority of the α-bungarotoxin insensitive α3, β2 subtype. However, Keyser et al. (1988) and Hamassaki-Britto et al. (1994) found α3 and β2 subunits to be present in amacrine, ganglion and displaced ganglion cells. This picture is complicated by the finding that cytisine was both potent (158 times > nicotine) and efficacious in evoking spreading depression, as this compound is relatively ineffective at nicotinic acetylcholine receptors containing the β2 subunit. In contrast receptors that contain the β4 subunit are more sensitive to cytisine than any other agonist (Luetje and Patrick, 1991; Chavez-Noriega et al., 1997) the α7 subunit is also sensitive to cytisine (Chavez-Noriega et al., 1997) and has been found in the chick retina (Hamassaki-Britto et al., 1994), however, this subunit is α-bungarotoxin sensitive. Thus it seems likely that a receptor containing the β4 subunit may be involved in these responses. The data suggest therefore that spreading depression produced by nicotine and cytisine in the chicken retina is evoked by α-bungarotoxin insensitive nicotinic acetylcholine receptor subtypes; placed in the inner plexiform or ganglion cell layers. These nicotinic receptor evoked spreading depressions were also inhibited in a concentration dependent fashion by the NMDA receptor antagonist CPP. This data is in agreement with the findings of Sheardown (1993) who showed that spreading depressions. triggered by NMDA, kainate, quisqualate, AMPA, and potassium chloride could all be inhibited by selective NMDA receptor antagonists suggesting that spreading depression in the chick retina is dependent on activation of glutamatergic NMDA receptors. Therefore it seems possible that nicotine triggers spreading depression by evoking release of glutamate which acts at the NMDA receptors. It has been shown by McGehee et al. (1995) that nicotine can enhance excitatory transmission through an increase in pre-synaptic calcium permeability, this effect was however α-bungarotoxin sensitive,

possibly involving $\alpha 7$ subunits, which appears not to be the case in the present study. Nicotine and cytisine have also been shown to elicit glutamate release in the spinal chord of the rat, using microdialysis techniques (Khan et al., 1996). Nicotine evoked spreading depression was blocked by the addition of cadmium chloride to the medium. This suggests the involvement of voltage operated calcium channels (VOCs) in the nicotine evoked glutamate release. Cadmium does not inhibit the triggering of spreading depression by excitatory amino acid agonists or potassium chloride (Sheardown, 1993), indicating a unique mode of action for nicotine in this respect. Cadmium is, however, a non-specific VOC blocker and further studies using specific blockers of L, N, and P type calcium channels are required to elucidate which channels are involved.

In conclusion, spreading depression can be evoked by nicotinic acetylcholinergic receptor agonists in the chicken retina. This effect is not α-bungarotoxin dependent, is indirect, being ultimately mediated by NMDA receptor activation and is sensitive to blockade of VOCs. Spreading depression has been proposed to be a putative mechanism for the triggering of classical migraine (Lauritzen, 1987) and to be a contributor to neuronal damage following cerebral ischaemia (Nedergaard, 1996), suggesting that intake of nicotine through smoking or nicotine patches may be associated with a risk of increased levels of neuronal damage and thus a worse clinical outcome following a stroke. Furthermore the triggering of spreading depression by nicotinic agonists may prove to be a useful model for the study of nicotine evoked glutamate release especially if the receptor subtype involved can be identified.

Acknowledgements

The author is grateful for the expert technical assistance of Anne Meincke, Lone Igel and Iben Bredmose.

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