ELSEVIER

European Journal of Pharmacology 370 (1999) 101–107

ejp

Cholinergic modulation of the acoustic startle response in the caudal pontine reticular nucleus of the rat

Markus Fendt *, Michael Koch

Tierphysiologie, Universität Tübingen, Auf der Morgenstelle 28, D-72076 Tübingen, Germany

Received 30 November 1998; received in revised form 22 February 1999; accepted 2 March 1999

Abstract

The startle response is a useful behavioural model to assess drug effects on sensorimotor information processing in the mammalian central nervous system. Prepulse inhibition of the acoustic startle response in rats is an operational measure for sensorimotor gating mechanisms which may be necessary for attention and response selection. The caudal pontine reticular nucleus is a key element of the pathway that mediates the acoustic startle response and receives an inhibitory cholinergic projection that might be important for prepulse inhibition. The present study tested whether prepulse inhibition of acoustic startle is modulated by microinfusions of the muscarinic/nicotinic acetylcholine receptor agonist carbachol and of the muscarinic acetylcholine receptor antagonist scopolamine. Carbachol (0–40 nmol/0.5 μl) dose dependently attenuated startle and enhanced prepulse inhibition. Scopolamine (0–40 nmol/0.5 μl) dose-dependently enhanced startle and reduced prepulse inhibition at a dose of 10 nmol. Scopolamine (40 nmol) also increased the spontaneous motor activity of the rats. These findings lend support to the hypothesis that muscarinic acetylcholine receptors in the caudal pontine reticular nucleus inhibit the acoustic startle response and are involved in the mediation of prepulse inhibition of startle.

Keywords: Acetylcholine; Acoustic startle response; Carbachol; Prepulse inhibition; (Rat); Scopolamine

1. Introduction

The startle response in rats is a useful behavioural tool for the investigation of sensorimotor information processing and its modulations in mammals (reviewed in Davis et al., 1993; Yeomans and Frankland, 1996; Koch and Schnitzler, 1997). The acoustic startle response is elicited by sudden intense acoustic stimuli and is composed of a twitch of facial, neck and limb muscles (Landis and Hunt, 1939). In the rat, the acoustic startle response is mediated by a pathway comprising the cochlear root nucleus and the caudal pontine reticular nucleus which projects onto facial, cranial and spinal motor neurones (Lee et al., 1996; Yeomans and Frankland, 1996; Koch and Schnitzler, 1997).

The caudal pontine reticular nucleus also receives projections from other brain areas that modulate the acoustic startle response, for example, from the periaqueductal grey, the amygdala and various tegmental nuclei which enhance startle (Koch and Schnitzler, 1997). The projection from the pedunculopontine tegmental nucleus to the caudal pontine reticular nucleus is part of an inhibitory pathway which mediates the reduction in magnitude of the acoustic startle response produced by a weak prepulse presented 30–500 ms before the startle stimulus, a phenomenon termed prepulse inhibition (Hoffman and Ison, 1980). Prepulse inhibition is widely used as an operational measure of sensorimotor gating mechanisms which are important for the suppression of inadvertent sensory or motor events (reviewed in Swerdlow et al., 1992; Koch and Schnitzler, 1997). Since impaired prepulse inhibition is found in humans in some neuropsychiatric disorders, including schizophrenia (Swerdlow and Geyer, 1998), there is considerable interest in the mechanisms that mediate and regulate prepulse inhibition in rats.

Lesions of the pedunculopontine tegmental nucleus reduce prepulse inhibition (Koch et al., 1993; Swerdlow and Geyer, 1993; Kodsi and Swerdlow, 1997). Anatomical and electrophysiological studies revealed that acetylcholine is one of the transmitters of the projection from the pedunculopontine tegmental nucleus to the caudal pontine reticular nucleus and exerts an inhibitory effect on the tone-evoked

* Corresponding author. Tel.: +49-7071-2975347; Fax: +49-7071-292618; E-mail: markus.fendt@uni-tuebingen.de

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00156-9

activity of acoustically responsive caudal pontine reticular nucleus neurones (Koch et al., 1993; Kungel et al., 1994). Excitotoxic lesions that preferentially destroyed cholinergic pedunculopontine tegmental nucleus neurones significantly reduced prepulse inhibition, suggesting that acetylcholine is the transmitter that conveys the inhibitory effect of auditory prepulses on the acoustic startle response onto the caudal pontine reticular nucleus (Koch et al., 1993; Koch and Schnitzler, 1997).

Acetylcholine in the medial pontine reticular formation has already been shown to be important for the regulation of REM (rapid eye movement) sleep in cats and rats (Baghdoyan et al., 1984; Imeri et al., 1994; Leonard and Llinas, 1994; see Rye, 1997 for a recent review). The most prominent finding of these studies being that the local microinjection of the muscarinic acetylcholine receptor agonist carbachol into different sites in the pontine reticular formation induces REM sleep (but see Deurveilher et al., 1997).

Studies using systemic application of cholinergic drugs have not yielded a clear picture of the role of acetylcholine in the regulation of startle (Davis, 1980). The present study tested the hypothesis that muscarinic acetylcholine receptors in the caudal pontine reticular nucleus are involved in the inhibition of the acoustic startle response. Therefore, we injected different doses of the acetylcholine receptor agonist carbachol, as well as the acetylcholine receptor antagonist scopolamine into the caudal pontine reticular nucleus and tested the effect of these drugs on the acoustic startle response, on spontaneous motor activity and on acoustic prepulse inhibition of startle.

2. Materials and methods

2.1. Animals

Thirty-six experimentally naive male Sprague–Dawley rats (Charles River, Sulzfeld Germany), weighing 240–340 g at the beginning of the experiments, were used. They were housed in groups of 4–6 under a continuous light/dark cycle (lights on from 0700 to 1900 h). The rats received 12 g rat chow per animal per day, and water was freely available. The experiments were done in accordance with ethical guidelines for the care and use of experimental animals and were approved by the local council of animal care (Regierungspräsidium Tübingen, ZP 4/96).

2.2. Surgery

The rats were anaesthetised with chloral hydrate (420 mg/kg) and placed into a stereotaxic frame. Two 22-gauge stainless steel guide cannulae were implanted bilaterally into the brain aiming at the caudal pontine reticular nucleus. The following stereotaxic coordinates from the atlas of Paxinos and Watson (1997) were used: rostrocaudal −9.0 mm; mediolateral ±1.5 mm; dorsoventral 9.9 mm (relative to Bregma, incisor bar −3.3 mm below the interaural line). The cannulae were fixed to the skull with dental cement and three anchoring screws. After surgery and between the experiments, stylets were inserted into the guide cannulae to maintain patency. The animals were tested after 5 days of recovery from surgery.

2.3. Testing procedure

To inject the different doses of carbachol (carbamylcholine chloride; Sigma, Deisenhofen, Germany) and scopolamine ((−)-scopolamine methylbromide; Sigma, Deisenhofen, Germany), 27-gauge stainless steel injection cannulae were inserted into the guide cannulae. Drugs or vehicle (0.9% NaCl) were administered in an injection volume of 0.5 μl within 60 s and the injection cannulae were left in place for further 120 s to allow diffusion of the drugs into the caudal pontine reticular nucleus. Each rat received only one drug (carbachol or scopolamine). A within-subjects design was used, so that each rat received each concentration (0, 10, 20, 30 and 40 nmol/0.5 μl) of a drug according to a latin square design on each of 5 consecutive days with 24 h between each injection.

To test the acoustic startle response amplitude, prepulse inhibition and the spontaneous motor activity, the rats were placed into two identical wire mesh cages ($20 \times 10 \times 12$ cm^3) with a steel floor, which were placed on two piezoelectric accelerometers (custom-made at the University of Tübingen). The accelerometers were located inside two sound-attenuated test chambers ($100 \times 80 \times 60$ cm^3). Movements of the rats resulted in changes of the voltage output of the accelerometers. These signals were amplified, digitised and fed into a data-acquisition board in a computer for further analysis. A loudspeaker, set up at a distance of 40 cm away from the test cage, delivered the acoustic startle stimuli and a continuous white background noise (55 dB sound pressure level). Intensity measurements were done with a 1/2 in. condenser microphone and a measuring amplifier (Brüel and Kjaer, Copenhagen, Denmark) after filtering (band-pass: 0.25–80 kHz). The presentation of the acoustic stimuli was controlled by a computer and an appropriate interface (Hortmann universal function synthesiser, Hortmann, Neckartenzlingen, Germany). The whole body startle amplitude was calculated from the difference between the peak-to-peak voltage output of the accelerometer within time-windows of 80 ms after and 80 ms before the startle stimulus onset. The spontaneous motor activity was calculated as the root mean square value of the accelerometer output, measured in a time window of 28 s before the presentation of each test trial.

After an acclimation time of 5 min, the test session began with an initial startle stimulus followed by four different trial types presented in a random order with an interstimulus interval of 30 s. Each trial type was presented

five times: (1) acoustic startle stimulus alone (white noise, 100 dB sound pressure level, 2×10^{-5} N/m, 20 ms duration, 0 ms rise time), (2) acoustic startle stimulus preceded by a prepulse (70 dB sound pressure level, 10 kHz, 20 ms duration including rise/fall times of 0.4 ms, 100 ms before onset of the startle stimulus), (3) prepulse alone, (4) no stimulus.

2.4. Histology and statistical analysis

After the tests the rats were killed by an overdose of pentobarbital (Nembutal), the brains were removed from the skull and immersion-fixed with 8% paraformaldehyde in phosphate-buffered saline with 20% sucrose. Coronal sections of 60 μm were taken on a freezing microtome and stained with thionine. The injections sites were plotted onto plates taken from the atlas of Paxinos and Watson (1997).

For statistical analysis, the acoustic startle response amplitudes of each trial type were averaged and are presented as mean ± S.E.M. Prepulse inhibition was the percent decrease of the acoustic startle response following a startle stimulus preceded by a prepulse compared to the acoustic startle response without prepulse [100 × (mean amplitude on startle stimulus alone − mean amplitude on startle stimulus preceded by a prepulse)/mean amplitude on startle stimulus alone]. The treatment effects on startle amplitude, percent prepulse inhibition and spontaneous motor activity were evaluated using repeated measures analysis of variance (ANOVA) followed by post hoc Tukey's *t*-test for pairwise comparison. A value of $P < 0.05$ was taken as the criterion for statistical significance.

3. Results

3.1. Histology

Histological analysis revealed that 28 rats received bilateral injections of carbachol ($n = 13$) or scopolamine ($n = 15$) mainly into the lateral part of the caudal pontine reticular nucleus (injection sites are shown in Fig. 1). The injection sites of the remaining eight rats were located outside the caudal pontine reticular nucleus. These cases were excluded from further analysis.

3.2. Possible effects of testing procedures on baseline startle amplitude, prepulse inhibition and spontaneous motor activity

An ANOVA revealed that there was an effect of the test day of saline injections on the baseline startle magnitude (F's > 3.01; p's < 0.04) reflecting long-term habituation. To exclude possible carry-over effects of carbachol and scopolamine, we further tested whether the baseline startle magnitudes, prepulse inhibition and spontaneous motor activity after saline injections depend on the drug dose given at the previous day. No effect was found (ANOVA: F's < 1.10; P's > 0.29) so that it was possible to pool the test values of the different test days.

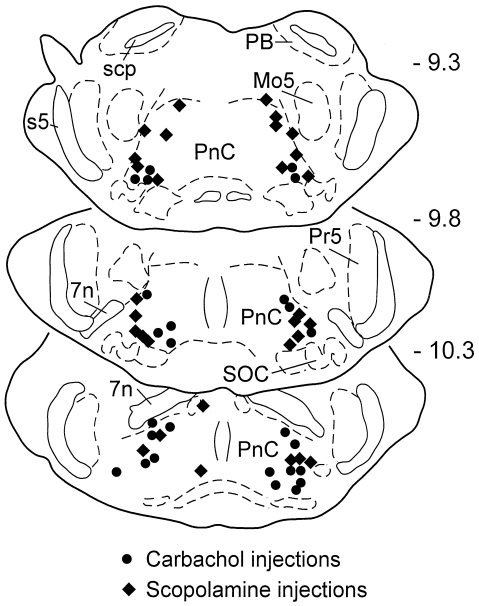

Fig. 1. Serial drawings of coronal sections through the lower brainstem depicting the injection sites of carbachol ($n = 13$; filled circles) and scopolamine ($n = 15$; filled diamonds) into the caudal pontine reticular nucleus. Abbreviations: 7n, facial nerve; Mo5, motor trigeminal nucleus; PB, parabrachial complex; PnC, caudal pontine reticular nucleus; Pr5, principal sensory trigeminal nucleus; s5, sensory root of the trigeminal nerve; scp, superior cerebellar peduncle; SOC, superior olivary complex. Numerals indicate the distance from Bregma (in mm).

The rats of the two experimental groups (carbachol and scopolamine) showed no differences with respect to the basal startle magnitude (Student's *t*-test: $t = -1.10$, $P = 0.30$), prepulse inhibition ($t = 1.71$, $P = 0.11$) and spontaneous motor activity ($t = 0.81$, $P = 0.43$) after injections of saline.

3.3. Injections of carbachol

Fig. 2 depicts the effect of carbachol injections into the caudal pontine reticular nucleus on the basal startle magnitude, i.e., the pulse-alone trials (A), on percent prepulse inhibition, i.e., prepulse–pulse trials (B), and on spontaneous motor activity (C). The basal startle magnitude was dose-dependently attenuated after injections of carbachol (ANOVA: $F(4,64) = 5.25$, $P = 0.001$; Fig. 2A). Post hoc Tukey's *t*-tests revealed a significant difference between the effect on startle magnitude of saline injections and injections of 20 nmol carbachol ($t = 2.93$, $P < 0.01$), 30 nmol carbachol ($t = 3.37$, $P < 0.01$), and 40 nmol carbachol ($t = 4.14$, $P < 0.01$).

For the statistical analysis of the carbachol effects on prepulse inhibition, the prepulse inhibition value after in-

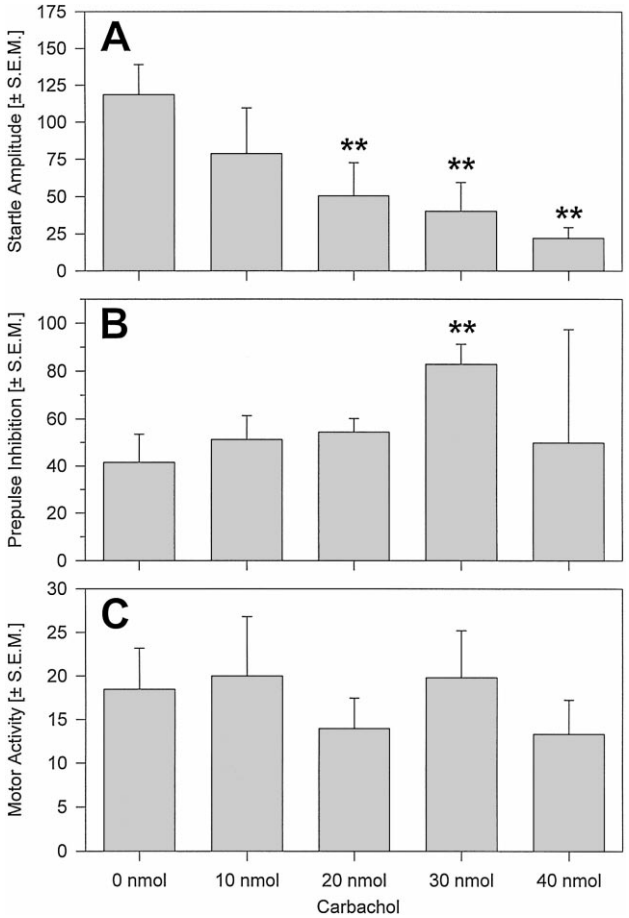

Fig. 2. Bar diagram depicting the mean acoustic startle response amplitude in pulse-alone trials (A), percent prepulse inhibition (B) and spontaneous motor activity (C) in arbitrary accelerometer readings after injections of 0, 10, 20, 30 and 40 nmol carbachol into the caudal pontine reticular nucleus ($n = 13$). $^{**}P < 0.01$ for the comparison between saline and carbachol injections (post hoc *t*-Tukey's tests after a significant main effect of the ANOVA).

jections of 40 nmol carbachol were excluded (but shown in Fig. 2B). The reason for this was that half of the animals showed no startle amplitude after injections of 40 nmol carbachol (i.e., amplitudes < 10 arbitrary startle units), so that an inclusion of the corresponding prepulse inhibition scores into the statistical analysis was not reliable due to the large scatter. Injections of carbachol into the caudal pontine reticular nucleus dose-dependently enhanced prepulse inhibition of the acoustic startle response (ANOVA: $F(3,36) = 3.46$, $P = 0.026$; Fig. 2B). Post hoc Tukey's *t*-tests revealed a significant difference between 0 nmol and 30 nmol carbachol ($t = -3.09$; $P < 0.01$). No significant effects on prepulse-alone trials were found (ANOVA: $F(4,64) = 1.24$; $P = 0.30$; data not shown).

Carbachol injections into the caudal pontine reticular nucleus had no effect on the spontaneous motor activity in the test cage (ANOVA: $F(4,64) = 0.59$; $P = 0.67$; Fig. 2C).

3.4. Injections of scopolamine

Fig. 3 shows the effects of scopolamine injections into the caudal pontine reticular nucleus on the basal startle magnitude (A), on prepulse inhibition (B) and on spontaneous motor activity (C). Injections of scopolamine increased the basal startle magnitude (ANOVA: $F(4,56) = 5.57$, $P = 0.0008$; Fig. 3A). Post hoc Tukey's *t*-tests revealed significant differences between injections of saline and injections of 20 nmol ($t = -3.30$, $P < 0.05$), 30 nmol ($t = -3.17$, $P < 0.01$) and 40 nmol scopolamine ($t = -2.94$, $P < 0.05$).

The statistical analysis of the percent prepulse inhibition scores showed only a trend of reduction after injections of scopolamine into the caudal pontine reticular nucleus (ANOVA: $F(4,56) = 2.02$, $P = 0.10$; Fig. 3B). Student's *t*-tests revealed a significant attenuation of percent prepulse inhibition after injections of saline and 10 nmol

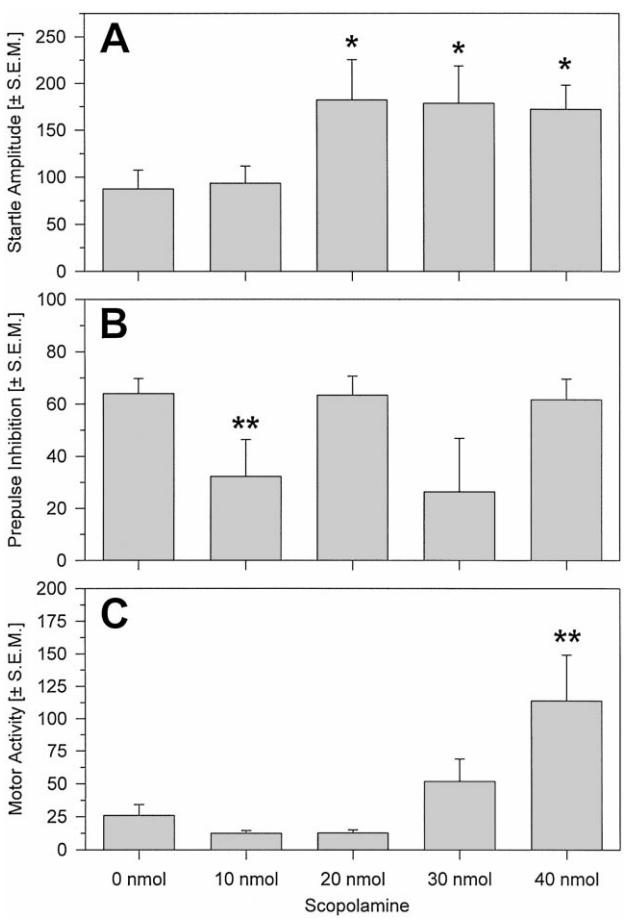

Fig. 3. Bar diagram depicting the mean acoustic startle response amplitude in pulse-alone trials (A), prepulse inhibition (B) and spontaneous motor activity (C) in arbitrary accelerometer readings after injections of 0, 10, 20, 30 and 40 nmol scopolamine into the caudal pontine reticular nucleus ($n = 15$). $^{*}P < 0.05$, $^{**}P < 0.01$ for the comparison between saline and carbachol injections (post hoc *t*-Tukey's tests after a significant main effect of the ANOVA).

scopolamine ($t = 2.11$, $P = 0.002$). The other doses of scopolamine did not affect prepulse inhibition (P's > 0.05). No significant effects on prepulse-alone trials were found (ANOVA: $F(4,56) = 0.82$; $P > 0.52$; data not shown).

Injections of scopolamine into the caudal pontine reticular nucleus significantly increased the spontaneous motor activity in the test cage (ANOVA: $F(4,56) = 6.54$, $P = 0.0002$). Post hoc Tukey's t-tests revealed a significant difference between spontaneous motor activity after injections of saline and 40 nmol scopolamine ($t = -3.73$, $P < 0.01$). After injections of this high dose of scopolamine, spontaneous body twitches, akin to stimulus-induced startle responses, were observed.

4. Discussion

The present study tested the hypothesis that acetylcholine receptors in the caudal pontine reticular nucleus are involved in the modulation of the acoustic startle response in rats. This hypothesis was supported by the finding that injections of the acetylcholine receptor agonist carbachol dose-dependently decreased the baseline startle amplitude, whereas injections of the acetylcholine receptor antagonist scopolamine increased the basal startle magnitude. Moreover, prepulse inhibition of startle was enhanced by carbachol injections and attenuated by injections of 10 nmol scopolamine. The spontaneous motor activity in the test cage was not affected by carbachol, but increased by scopolamine injections into the caudal pontine reticular nucleus.

Our data strongly suggest that acetylcholine transmission in the caudal pontine reticular nucleus inhibits the baseline startle magnitude. This confirms previous studies of our laboratory showing that microiontophoretical application of the predominantly muscarinic acetylcholine receptor agonists carbachol and acetyl-β-methylcholine into the caudal pontine reticular nucleus inhibit the tone-evoked activity of caudal pontine reticular nucleus neurones measured by extracellular recordings (Koch et al., 1993; Kungel et al., 1994). This inhibitory effect was observed in about 70% of the acoustically responsive caudal pontine reticular nucleus neurones. The remaining 30% of the these neurones were excited by acetylcholine. Approximately 45% of the non-acoustically responsive caudal pontine reticular nucleus neurones were inhibited and 45% were excited by the acetylcholine agonists. Anatomical tracing studies showed that the pedunculopontine tegmental nucleus is the origin of a cholinergic projection to the caudal pontine reticular nucleus (Semba et al., 1990; Koch et al., 1993). Lesions of the pedunculopontine tegmental nucleus enhanced the basal startle magnitude (Swerdlow and Geyer, 1993) and attenuated prepulse inhibition of the acoustic startle response (Koch et al., 1993; Swerdlow and Geyer, 1993; Kodsi and Swerdlow, 1997).

Due to these facts, we expected that local injections of carbachol and scopolamine into the caudal pontine reticular nucleus affect prepulse inhibition. In the present study, local injections of carbachol into the caudal pontine reticular nucleus dose-dependently enhanced prepulse inhibition but injections of scopolamine did only attenuate prepulse inhibition in a dose of 10 nmol. This non-linear dose–response curve of scopolamine demands a cautious interpretation of our data. We have no explanation for the observation that scopolamine at higher doses does not block prepulse inhibition. There might be competing cholinergic effects within the caudal pontine reticular nucleus, or carbachol and scopolamine might affect different types of muscarinic receptors. Furthermore, the pronounced increase of the basal startle magnitude and spontaneous motor activity (which was observed after infusions of the higher doses of scopolamine) may interfere with the effects of scopolamine on prepulse inhibition.

It is noteworthy that the scopolamine injections in the present study enhanced the spontaneous motor activity of the rats. Interestingly, spontaneous motor twitches could be observed in rats after injections of the highest dose of scopolamine suggesting that disinhibition of the caudal pontine reticular nucleus by scopolamine leads to startle-like responses even in the absence of startling stimuli. Together with the effects of scopolamine injections on the baseline startle amplitude, these results suggest that acetylcholine in the pontine reticular formation generally exerts a tonic inhibitory influence on premotor neurones. Although piezoelectric accelerometers are able to measure changes in spontaneous motor activity (i.e., this study; McNish et al., 1997), we did not find an effect of carbachol injections into the caudal pontine reticular nucleus on spontaneous motor activity.

Acetylcholine in the pontine reticular formation is involved in the regulation of REM sleep (reviewed in Siegel, 1979; Baghdoyan et al., 1984; Gnadt and Pegram, 1986; Imeri et al., 1994; Leonard and Llinas, 1994; Rye, 1997; Scarnati and Florio, 1997; Hobson et al., 1998), so that it might be conceived that carbachol infusions in our study affected the startle response by changing the state of arousal of the rats. However, those brain sites where REM sleep is reliably evoked by carbachol are located further rostral and dorsomedial than our injection sites, so that possibly confounding effects on startle and prepulse inhibition of changes in the vigilance of the rats can be excluded.

The present study was based on results from previous experiments that lead to the hypothesis that the transmitter acetylcholine in the caudal pontine reticular nucleus is involved in the mediation of prepulse inhibition (Koch et al., 1993; Koch and Schnitzler, 1997). The caudal pontine reticular nucleus, especially its ventrolateral part (Lee et al., 1996), is a key element of a serial pathway that mediates the acoustic startle response. There is a cholinergic projection from the pedunculopontine tegmental nu-

cleus to the caudal pontine reticular nucleus (Semba et al., 1990; Koch et al., 1993) and extracellular recordings of caudal pontine reticular nucleus neurones showed a predominantly inhibitory effect of acetylcholine (Koch et al., 1993; Kungel et al., 1994).

Lesions of the pedunculopontine tegmental nucleus attenuated prepulse inhibition of the acoustic startle response (Koch et al., 1993; Swerdlow and Geyer, 1993; Kodsi and Swerdlow, 1997) and enhanced the basal startle magnitude (Swerdlow and Geyer, 1993). Based on these data, Koch and Schnitzler (1997) presented a hypothetical neuronal circuitry mediating acoustic prepulse inhibition. This model posits that the prepulse is processed in the ascending auditory system (i.e., cochlear nuclei, nucleus of the lateral lemniscus, superior olivary complex, inferior colliculus) and activates the inhibitory cholinergic projection from the pedunculopontine tegmental nucleus to the primary startle pathway via the superior colliculus. The activation of this inhibitory tegmentopontine loop is hypothesised to protect the prepulse processing from disruption by the sensory and motor events associated with the acoustic startle response. The present finding that the acetylcholine receptor agonist carbachol in the caudal pontine reticular nucleus inhibits the acoustic startle response and enhances prepulse inhibition, whereas injections of the acetylcholine receptor antagonist scopolamine increases the acoustic startle response and attenuates prepulse inhibition lends support to this hypothetical pathway. Yet, it is important to note that scopolamine did not completely block prepulse inhibition.

There might be additional pathways that mediate prepulse inhibition. For example, projections of the lateral superior olive and ventral nucleus of the trapezoid body change the excitability of the outer and inner hair cells via the olivary cochlear bundle (White and Warr, 1983). Based on these facts, Davis suggested the possibility that the acoustic prepulse reduces the startle response by inhibiting primary auditory receptors (Michael Davis, personal communication). If this mechanism is not sensitive to cholinergic neurotransmission, this would explain why scopolamine did not totally block prepulse inhibition.

A series of earlier studies investigated the effects of systemic injections of cholinergic drugs on startle and its modulations (summarised in Davis, 1980). The data reviewed by Davis from experiments with systemic or intracerebroventricular administration of muscarinic cholinergic drugs largely confirm the present findings of an enhancement of startle by an acetylcholine receptor antagonist and an attenuation of startle by an acetylcholine receptor agonist after local injections into the primary startle pathway. Although it should be noted that some recent studies failed to show that systemic scopolamine injections affect the basal startle response (Wu et al., 1993; Koch, 1996) and also fear-potentiated startle (Davis et al., 1993). However, reduced prepulse inhibition was found in one of these studies after treatment with scopolamine or after maintaining rats on a choline-free diet (Wu et al., 1993). Hughes (1984) showed that systemic applications of different cholinergic drugs did not affect habituation, another phenomenon of attenuated startle. On the other hand, Acri (1994), Acri et al. (1994, 1995, 1998) reported that nicotine injections dose-dependently increase the basal startle magnitude. Furthermore, while chronically administered nicotine enhanced prepulse inhibition only in old but not in young rats, acute systemic injections of lower doses of nicotine increased prepulse inhibition (Acri et al., 1994, 1995). Chronic prenatal nicotine exposure resulted in an increase of nicotinic receptors in the brain and in an attenuation of prepulse inhibition in female rats (Popke et al., 1997). Nicotine increased prepulse inhibition and the nicotinic antagonist mecamylamine decreased prepulse inhibition, and both compounds had no effect on the basal startle magnitude (Curzon et al., 1994). Interestingly, an abstract by Sorenson and Wilkinson (1983) reported on a decrease of fear-potentiated startle after nicotine injections suggesting an anxiolytic action of nicotine. The partially contradicting findings concerning the role of acetylcholine for the regulation of the startle response might be explained by the fact that in different parts of the brain the distribution of pre- and postsynaptic acetylcholine receptors is different, so that the systemical application of cholinomimetics might affect different brain systems in different ways. For example, in the pontine reticular formation the release of acetylcholine is *increased* by low doses of the cholinergic antagonist scopolamine through the blockade of presynaptic muscarinic M_2 autoreceptors (Baghdoyan et al., 1998). Taken together with the present data it is evident that acetylcholine affects the acoustic startle response in various ways probably affecting pre- and postsynaptic receptors in different brain areas.

In summary, the present study shows that acetylcholine inhibits the acoustic startle response at the level of the primary startle circuit. Our data do not fully support the hypothesis that acetylcholine is necessary for prepulse inhibition of startle.

Acknowledgements

We are grateful to Ms. Isabel Schwienbacher and Mr. Björn Steiniger for running some of the test, as well as to Ms. Helga Zillus for her excellent technical assistance. This study was supported by the Deutsche Forschungsgemeinschaft (SFB 307/C2, Fe 483/1-1, SPP 1001, and a Heisenberg-fellowship to M.K.).

References

Acri, J.B., 1994. Nicotine modulates effects of stress on acoustic startle reflexes in rats: dependence on dose, stressor and initial reactivity. Psychopharmacology 116, 255–265.

Acri, J.B., Morse, D.E., Popke, E.J., Grunberg, N.E., 1994. Nicotine

increases sensory gating measured as inhibition of the acoustic startle reflex in rats. Psychopharmacology 114, 369–374.
Acri, J.B., Brown, K.J., Saah, M.I., Grunberg, N.E., 1995. Strain and age differences in acoustic startle responses and effects of nicotine in rats. Pharmacol. Biochem. Behav. 50, 191–198.
Acri, J.B., Grunberg, N.E., Morse, D.E., 1998. Effects of nicotine on the acoustic startle reflex amplitude in rats. Psychopharmacology 104, 244–248.
Baghdoyan, H.A., Rodrigo-Angulo, M.L., McCarley, R.W., Hobson, J.A., 1984. Site-specific enhancement and suppression of desynchronized sleep signs following cholinergic stimulation of three brainstem regions. Brain Res. 306, 39–52.
Baghdoyan, H.A., Lydic, R., Fleegal, M.A., 1998. M2 muscarinic autoreceptors modulate acetylcholine release in the medial pontine reticular formation. J. Pharmacol. Exp. Ther. 286, 1446–1452.
Curzon, P., Kim, D.J.B., Decker, M.W., 1994. Effect of nicotine, lobeline, and mecamylamine on sensory gating in the rat. Pharmacol. Biochem. Behav. 49, 877–882.
Davis, M., 1980. Neurochemical modulation of sensory–motor reactivity: acoustic and tactile startle reflexes. Neurosci. Biobehav. Rev. 4, 241–263.
Davis, M., Falls, W.A., Campeau, S., Kim, M., 1993. Fear-potentiated startle: a neural and pharmacological analysis. Behav. Brain Res. 58, 175–198.
Deurveilher, S., Hars, B., Hennevin, E., 1997. Pontine microinjection of carbachol does not reliably enhance paradoxical sleep in rats. Sleep 20, 593–607.
Gnadt, J.W., Pegram, G.V., 1986. Cholinergic brainstem mechanisms of REM sleep in the rat. Brain Res. 384, 19–41.
Hobson, J.A., Stickgold, R., Pace-Schott, E.F., 1998. The neuropsychology of REM sleep dreaming. NeuroReport 9, R1–R14.
Hoffman, H.S., Ison, J.R., 1980. Reflex modification in the domain of startle: I. Some empirical findings and their implications for how the nervous system processes sensory input. Psychol. Rev. 87, 175–189.
Hughes, R.N., 1984. Cholinergic and monoaminergic substrates of startle habituation. Life Sci. 34, 2101–2105.
Imeri, L., Bianchi, S., Angeli, P., Mancia, M., 1994. Selective blockade of different brain stem muscarinic receptor subtypes: effects on the sleep–wake cycle. Brain Res. 636, 68–72.
Koch, M., 1996. The septohippocampal system is involved in prepulse inhibition of the acoustic startle response in rats. Behav. Neurosci. 110, 468–477.
Koch, M., Schnitzler, H.-U., 1997. The acoustic startle response in rats—circuits mediating evocation, inhibition and potentiation. Behav. Brain Res. 89, 35–49.
Koch, M., Kungel, M., Herbert, H., 1993. Cholinergic neurons in the pedunculopontine tegmental nucleus are involved in the mediation of prepulse inhibition of the acoustic startle response in the rat. Exp. Brain Res. 97, 71–82.
Kodsi, M.H., Swerdlow, N.R., 1997. Regulation of prepulse inhibition by ventral pallidal projections. Brain Res. Bull. 43, 219–228.
Kungel, M., Ebert, U., Herbert, H., Ostwald, J., 1994. Substance P and other putative transmitters modulate the activity of reticular pontine neurons: an electrophysiological and immunohistochemical study. Brain Res. 643, 29–39.
Landis, C., Hunt, W.A., 1939. The Startle Pattern. Farrar and Rinehart, New York.
Lee, Y., Lopez, D.E., Meloni, E.G., Davis, M., 1996. A primary acoustic startle pathway: obligatory role of cochlear root neurons and the nucleus reticularis pontis caudalis. J. Neurosci. 16, 3775–3789.
Leonard, C.S., Llinas, R., 1994. Serotonergic and cholinergic inhibition of mesopontine cholinergic neurons controlling REM sleep: an in vitro electrophysiological study. Neuroscience 59, 309–330.
McNish, K.A., Gewirtz, J.C., Davis, M., 1997. Evidence of contextual fear after lesions of the hippocampus: a disruption of freezing but not fear-potentiated startle. J. Neurosci. 17, 9353–9360.
Paxinos, G., Watson, C., 1997. The Rat Brain in Stereotaxic Coordinates. Academic Press, San Diego.
Popke, E.J., Tizabi, Y., Rahman, M.A., Nespor, S.M., Grunberg, N.E., 1997. Prenatal exposure to nicotine: effects on prepulse inhibition and central nicotinic receptors. Pharmacol. Biochem. Behav. 58, 843–849.
Rye, D.B., 1997. Contributions of the pedunculopontine region to normal and altered REM sleep. Sleep 20, 757–788.
Scarnati, E., Florio, T., 1997. The pedunculopontine nucleus and related structures: functional organization. In: Obeso, J.A., DeLong, M.R., Ohye, C., Marsden, C.D. (Eds.), The Basal Ganglia and New Surgical Approaches for Parkinson's Disease, Advances in Neurobiology. Lippincott-Raven, Philadelphia, pp. 97–110.
Semba, K., Reiner, P.B., Fibiger, H.C., 1990. Single cholinergic mesopontine tegmental neurons project to both the pontine reticular formation and the thalamus in the rat. Neuroscience 38, 643–654.
Siegel, J.M., 1979. Behavioral functions of the reticular formation. Brain Res. Rev. 1, 69–105.
Sorenson, C.A., Wilkinson, L.S., 1983. Behavioral evidence that nicotine administration has anxiolytic actions. Soc. Neurosci. Abstr. 9, 137.
Swerdlow, N.R., Geyer, M.A., 1993. Prepulse inhibition of acoustic startle in rats after lesions of the pedunculopontine tegmental nucleus. Behav. Neurosci. 107, 104–117.
Swerdlow, N.R., Geyer, M.A., 1998. Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. Schizophr. Bull. 24, 285–301.
Swerdlow, N.R., Caine, S.B., Braff, D.L., Geyer, M.A., 1992. The neural substrates of sensorimotor gating of the startle reflex: a review of recent findings and their implications. J. Psychopharmacol. 6, 176–190.
White, J.S., Warr, W.B., 1983. The dual origins of the olivocochlear bundle in the albino rat. J. Comp. Neurol. 219, 203–214.
Wu, M.-F., Jenden, D.J., Fairchild, M.D., Siegel, J.M., 1993. Cholinergic mechanisms in startle and prepulse inhibition: effects of the false cholinergic precursor *N*-aminodeanol. Behav. Neurosci. 107, 306–316.
Yeomans, J.S., Frankland, P.W., 1996. The acoustic startle reflex: neurons and connections. Brain Res. Rev. 21, 301–314.