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The neuropeptide corticotropin-releasing factor regulates excitatory transmission and plasticity at the climbing fibre-Purkinje cell synapse

M. T. Schmolesky,* M. M. De Ruiter, C. I. De Zeeuw and C. Hansel
Department of Neuroscience, Erasmus University Medical Centre, Postbus 2040, 3000 CA Rotterdam, The Netherlands

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Abstract

The climbing fibre (CF) input controls cerebellar Purkinje cell (PC) activity as well as synaptic plasticity at parallel fibre (PF)-PC synapses. Under high activity conditions, CFs release not only glutamate, but also the neuropeptide corticotropin-releasing factor (CRF). Brief periods of such high CF activity can lead to the induction of long-term depression (LTD) at CF-PC synapses. Thus, we have examined for the first time the role of CRF in regulating excitatory postsynaptic currents (EPSCs) and long-term plasticity at this synapse. Exogenous application of CRF alone transiently mimicked three aspects of CF-LTD, causing reductions in the CF-evoked excitatory postsynaptic current, complex spike second component and complex spike afterhyperpolarization. The complex spike first component is unaffected by CF-LTD induction and was similarly unaffected by CRF. Application of a CRF receptor antagonist reduced the expression amplitude and induction probability of CF-LTD monitored at the EPSC level. Collectively, these results suggest that under particular sensorimotor conditions, co-release of CRF from climbing fibres could down-regulate excitatory transmission and facilitate LTD induction at CF-PC synapses. Inhibition of either protein kinase C (PKC) or protein kinase A (PKA) attenuated the effects of CRF upon CF-EPSCs. We have previously shown that CF-LTD induction is PKC-dependent, and here demonstrate PKA-dependence as well. These results suggest that both the acute effects of CRF on CF-EPSCs as well as the facilitating effect of CRF on CF-LTD induction can be explained by a CRF-mediated recruitment of PKC and PKA.

Introduction

Purkinje cells (PCs) are the only output neurons of the cerebellar cortex and play a central role in both motor control and the acquisition or modification of motor skills. PCs receive two forms of excitatory input: the climbing fibre (CF) projection from the inferior olive and the parallel fibre (PF) projections from granule cells. Each PC in the adult rat receives input from only one CF (Crepel *et al*., 1976), which is, nevertheless, potent because the CF forms ~1500 synapses upon the primary dendrite (Silver *et al*., 1998; Strata & Rossi, 1998). The PC response to CF stimulation is an all-or-none event, registered as an excitatory postsynaptic current (EPSC; in voltage-clamp) or a complex spike (in current-clamp) (see Fig. 1; Schmolesky *et al*., 2002, 2005). The CF maintains a unique heterosynaptic control of bidirectional synaptic plasticity at the PF input, by providing large dendritic calcium transients that favour long-term depression (LTD) over long-term potentiation (LTP) induction at PF synapses (Coesmans *et al*., 2004). Both PF and CF terminals release glutamate but CFs also contain the neuropeptide corticotropin-releasing factor (CRF). In fact, light and electron immunomicroscopy reveal a dense CRF label in inferior olive neurons, their axonal projections and the CF terminals (Palkovits *et al*., 1987; Tian & Bishop, 2003). Evidence suggests that CRF is released by CFs in an activity-dependent manner (Barmack & Young, 1990; Tian & Bishop, 2003) and may alter PC spike activity (Bishop, 1990; Bishop & King, 1992). CRF also impairs the PC slow afterhyperpolarization (AHP) following somatic current injection or complex spikes (Fox & Gruol, 1993; Miyata *et al*., 1999). Finally, CF-dependent release of CRF is required for the induction of LTD at the PF-PC synapse (Miyata *et al*., 1999). Despite these various reports on CRF actions, little is yet known about the role of CRF in modulating synaptic activity or plasticity at the CF-PC synapse itself. Recently, it has been shown that tetanic stimulation of the climbing fibre alone leads to LTD of the CF-PC synapse (Hansel & Linden, 2000; Carta *et al*., 2006). CF-LTD is expressed as a ~20% reduction in the CF-evoked EPSC, dendritic calcium transient, complex spike second component and AHP (Hansel & Linden, 2000; Weber *et al*., 2003; Schmolesky *et al*., 2005). CF-LTD is a postsynaptic phenomenon (Shen *et al*., 2002), and its induction is dependent upon activation of group I metabotropic glutamate receptors (mGluRs), a rise in cytosolic Ca^{2+} and protein kinase C (PKC) activation (Hansel & Linden, 2000). In this study we recorded from Purkinje cells in the *in vitro* cerebellar slice preparation to test the hypothesis that CRF plays a role in regulating activity and/or plasticity at the CF-PC synapse.

Materials and methods

Slice preparation and electrophysiology

Parasagittal slices of the cerebellar vermis (200 μm thick) were prepared from postnatal day 16–28 Sprague–Dawley rats. Rats were anaesthetized with isoflurane and decapitated in conformity with

Correspondence: Dr C. Hansel, as above.
E-mail: c.hansel@erasmusmc.nl

**Present address*: Department of Psychology, Weber State University, Ogden, Utah, USA.

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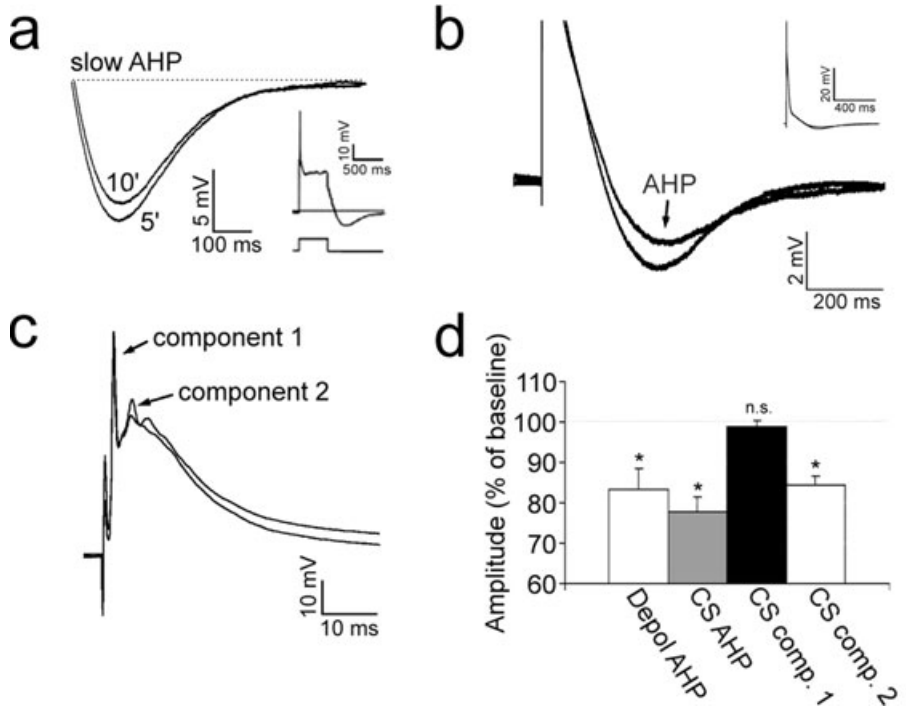

FIG. 1. CRF attenuates the Purkinje cell AHP and second complex spike component. Purkinje cell responses to somatic current injection or climbing fibre stimlation before (minute 5) and after (minute 10) CRF application. (a) Offset of a square wave depolarizing somatic current injection (inset) resulted in a slow PC afterhyperpolarization (Depol AHP) which was attenuated by focal application of CRF (1.0–1.5 μM). (b,c) Climbing fibre stimulation under current-clamp conditions resulted in complex spikes characterized by 2–6 fast spikes followed by a slow AHP. Focal application of CRF (1.0–1.5 μM) attenuated the complex spike AHP (b) and second component (c) while leaving the first component unaffected ($n = 3$). (d) Histogram demonstrating the effect of 5 min CRF upon Depol AHP and the complex spike components. *$P < 0.05$, compared with baseline. n.s. = not significant.

animal care protocols approved by the Erasmus University Medical Centre. Dissection and slicing were conducted in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 5 KCl, 1.25 Na_2HPO_4, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$ and 10 D-glucose bubbled with 95% O_2/5% CO_2. Slices were perfused with room-temperature ACSF supplemented with 20 μM picrotoxin to block γ-aminobutyric acid type A receptors (flow rate 1–3 mL/min). Whole-cell patch clamp recordings were conducted using a Zeiss Axioskop FS (Carl Zeiss, Göttingen, Germany) and EPC-9 amplifier (HEKA Electronics, Lambrecht/Pfalz, Germany). For current-clamp recordings, patch pipettes were filled with (in mM): 9 KCl, 10 KOH, 120 mM K gluconate, 3.48 $MgCl_2$, 10 HEPES, 4 NaCl, 4 Na_2-ATP, 0.4 mM Na_3-GTP and 17.5 sucrose (pH adjusted to 7.25). This same solution was used for LTD induction experiments (see Figs 3 and 5) where EPSC recordings were conducted in voltage-clamp mode and the 5-Hz, 30-s tetanus was delivered in current-clamp mode. For all other voltage-clamp experiments the pipette solution contained (in mM): 128 CsOH, 111 gluconic acid, 4 NaOH, 10 CsCl, 2 $MgCl_2$, 10 HEPES, 4 Na_2-ATP, 0.4 mM Na_3-GTP and 30 sucrose (pH adjusted to 7.25). Currents were filtered at 3 kHz, digitized at 8 kHz and acquired using PULSE software. The CF was stimulated using a stimulus isolation unit attached to a standard pipette filled with ACSF and placed in the granule cell layer. Test responses were evoked at 0.05 Hz with ~3.0-μA pulses of 0.5-ms duration. In voltage-clamp experiments, stimuli were delivered in pairs with a 50-ms separation to measure the paired pulse depression (PPD) ratio (EPSC2 amplitude/EPSC1 amplitude). Only cells that were innervated by a single CF, as indicated by a single step in the CF input–output relationship, were used. All drugs were purchased from Sigma, except CRF (Calbiochem, Nottingham, UK), astressin (Bachem, Weil am Rhein, Germany) and KT5720 (Tocris, Bristol, UK). Drugs were administered using a four-resevoir gravity-fed system with either a mini- or micromanifold (four-barreled 100-μm tip) for bath or focal application, respectively (BPS-4 system, ALA Systems).

Data analysis and inclusion criteria

Data analysis was conducted using PulseFit (HEKA) and Igor (Wavemetrics, Portland, OR, USA) software. A requirement for data inclusion was stability in resting membrane potential ($\Delta < \pm 5$ mV) and input resistance ($\Delta < \pm 15\%$) for current-clamp recordings or holding current, and input resistance ($\Delta < \pm 15\%$) and series resistance ($\Delta < \pm 15\%$) for voltage-clamp. Data for three sweeps collected per minute were averaged and normalized to the 5-min baseline. Group data are reported as mean ± SEM. For statistical analysis, one- and two-way ANOVAs were followed by planned *t*-tests (SPSS, Excel).

Results

CRF suppresses the Purkinje cell AHP and complex spike components

Somatic injection of depolarizing currents (500 ms; 200–600 pA) resulted in spike activity in PCs and, following current offset, a slow AHP (see Fig. 1a). Focal application of CRF for 5 min significantly reduced this AHP to 83 ± 5.5% of baseline values ($n = 10$, $P < 0.05$), but had no significant effect upon the resting membrane potential (−70.1 ± 1.5 mV at $t = 5$ min, −69.6 ± 1.8 mV at $t = 10$ min). Switching micropressure barrels from saline to an identical saline did not affect PC responses at the pressures used. On average, AHP suppression reached saturation within 5 min of CRF application, was

maintained until drug cessation and, at that time, showed reversal for some cells. These data extend the results of Fox & Gruol (1993) from cultured PCs to the cerebellar slice preparation where many of the PC synaptic connections are intact. CRF applied via bath perfusion also suppresses the slow AHP following complex spikes in PCs in cerebellar slices (Miyata *et al*., 1999). In our studies, focal application of CRF for 5 min to a single PC also significantly attenuated the complex spike AHP (77.6 ± 3.8%, $n = 3$, $P < 0.05$; Fig. 1b). Whereas Miyata *et al*. (1999) did not report on the early components of the complex spike, we found that the complex spike second component was simultaneously reduced by CRF (84.3 ± 2.4%, $P < 0.05$) while the first component was unaffected (99 ± 1.3%) (Fig. 1c and d). Evidence suggests that modification of the complex spike following CF-LTD induction could be the direct result of AMPA receptor modification and/or internalization at the CF-PC synapse (see Schmolesky *et al*., 2005). If CRF application acts upon the complex spike via the same mechanism we would expect to see an impact of CRF on the CF-PC EPSC.

CRF attenuates the CF-PC EPSC

CF-evoked EPSCs were recorded from PCs in voltage-clamp mode. The holding potential (typically −30 to −5 mV) was selected to prevent complex spikes, isolate the AMPA-R gated current and optimize cellular response stability. In the control condition, the CF-EPSC amplitude remained constant for the duration of recording (101 ± 3.3%, $t = 25$ min, $n = 6$). As shown in Fig. 2, addition of CRF (1.0 μM) to the perfusion media for 10 min reduced the CF-EPSC amplitude by half (52 ± 11.6%, $t = 16$ min, $n = 7$; $P < 0.05$). The EPSC suppression was, on average, reversed by a 15-min washout period (96 ± 10.0%, $t = 30$ min, $n = 5$), indicating that CRF was not toxic in nature. Had the impact of CRF upon the CF-EPSC been through a presynaptic mechanism, we would have expected an alteration in the PPD ratio (Hashimoto & Kano, 1998; Silver *et al*., 1998). Instead, the PPD ratio was not affected by CRF (Fig. 2d; 0.68 ± 0.03 at $t = 5$ min vs. 0.67 ± 0.04 at $t = 16$ min, $n = 7$, $P > 0.05$), suggesting a postsynaptic mechanism for CRF action upon EPSC amplitude.

CRF antagonist blocks CF-LTD induction

Bath application of the high-affinity CRF receptor antagonist astressin (0.2 μM) for 10 min had no effect upon the CF-EPSC (100 ± 1.3%, $t = 15$ min, $n = 4$) but completely blocked the effects of co-applied 1.0 μM CRF (97 ± 1.1%, $t = 25$ min; Fig. 3a). Under normal saline conditions, CF tetanization (5 Hz, 30 s) reduced the CF-EPSC within several minutes and this reduction reached a maximum within 15–20 min after tetanization (82 ± 3.5%, $t = 25$ min, $n = 9$, $P < 0.05$; Fig. 3b). Bath application of astressin (0.2 μM) for 15 min, beginning 10 min before CF tetanization, blocked the induction of CF-LTD at early and late (95 ± 1.8%, $t = 25$ min, $n = 13$; $P < 0.05$) time points (Fig. 3b). The induction of CF-LTD was considered successful when a cell demonstrated >10% reduction 20–25 min after CF tetanization. CF-LTD was induced in seven of nine cells in the control condition but only one of 13 cells in the astressin condition (Fig. 3c).

Kinase inhibitors block the impact of CRF upon the CF-PC EPSC

CRF receptors are G-protein bound and are reported to act through PKA and PKC (Blank *et al*., 2003). Thus, we examined the effects of kinase inhibitors upon the CRF-mediated suppression of the CF-PC EPSC. The PKC inhibitor chelerythrine (10 μM) was bath applied for 25–35 min prior to, and during, bath application of CRF (1 μM). In separate experiments, the PKA inhibitor KT5720 (0.1 μM) was bath applied for 10 min prior to, and for 10 min during, CRF application. As shown in Fig. 4, antagonism of PKC activity reduced the effects of CRF by approximately half compared with the CRF alone condition (72 ± 11.2%, $t = 16$ min, $n = 7$, $P = 0.05$), while antagonism of PKA activity completely blocked the effects of co-applied CRF (96 ± 3.6%, $t = 16$ min, $n = 7$, $P < 0.05$).

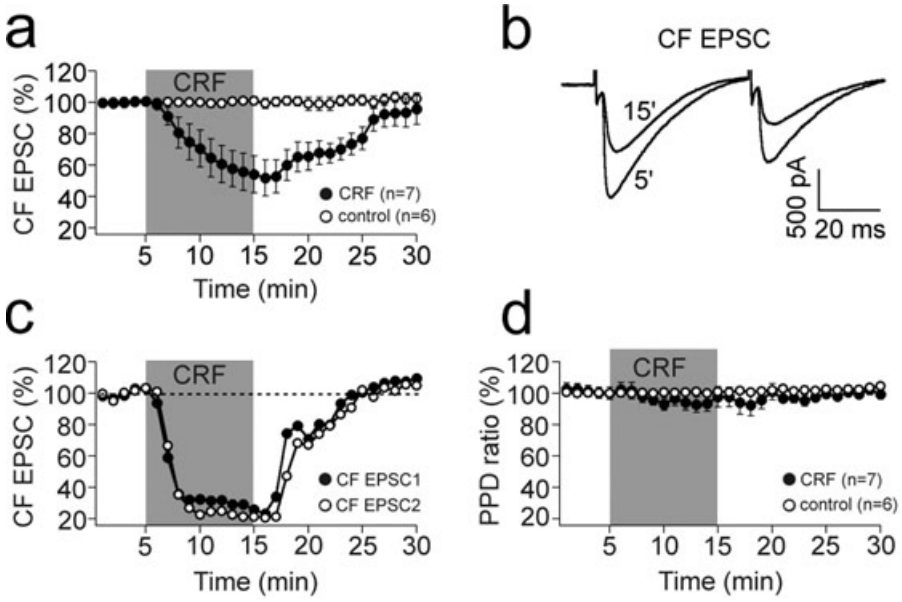

FIG. 2. CRF reduces the climbing fibre-Purkinje cell EPSC by half. (a) Bath application of CRF (1.0 μM) reduced the climbing fibre-evoked PC excitatory postsynaptic current. Washout led to a near complete recovery. (b) Representative CF-EPSCs just before CRF application (minute 5) and washout (minute 15). (c) Responses of an individual cell plotted across time show that the CRF induced reduction of the CF-EPSC was often swift and dramatic. (d) The paired pulse depression (PPD) ratio was not significantly affected by CRF application.

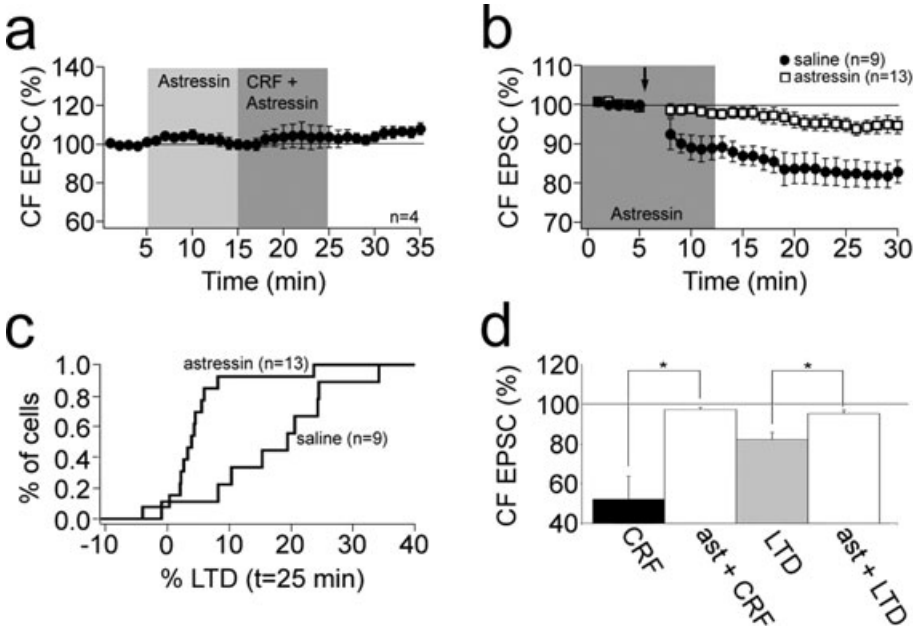

FIG. 3. CRF receptor antagonist blocks climbing fibre-Purkinje cell LTD. (a) CRF receptor antagonist astressin (0.2 μM) had no impact upon the CF-evoked Purkinje cell EPSC but completely blocked the effect of bath-applied CRF (1.0 μM, $n = 4$). (b) Application of astressin beginning 10 min prior to CF tetanization (5 Hz, 30 s; arrow) blocked LTD induction. (c) Cumulative probability distribution plotting the percentage of cells showing a given percentage of EPSC reduction at $t = 25$ min. The probability of LTD induction was greatly reduced in the CRF antagonist condition. (d) Summary histogram showing that the CRF antagonist astressin was sufficient to block the effect of 10 min CRF (1.0 μM) application and sufficient to block CF-LTD (minute 25). *$P < 0.05$, comparing conditions.

The induction of CF-LTD is PKA-dependent

The PKC- and PKA-dependence of the CRF-mediated reduction in CF-EPSC amplitudes led us to the question of whether these kinases are involved in CF-LTD induction as well, which would suggest that the acute effects of CRF signalling are related to the effects on CF-LTD induction. Indeed, as reported earlier, CF-LTD induction is dependent on the activation of PKC (Hansel & Linden, 2000). To test for the

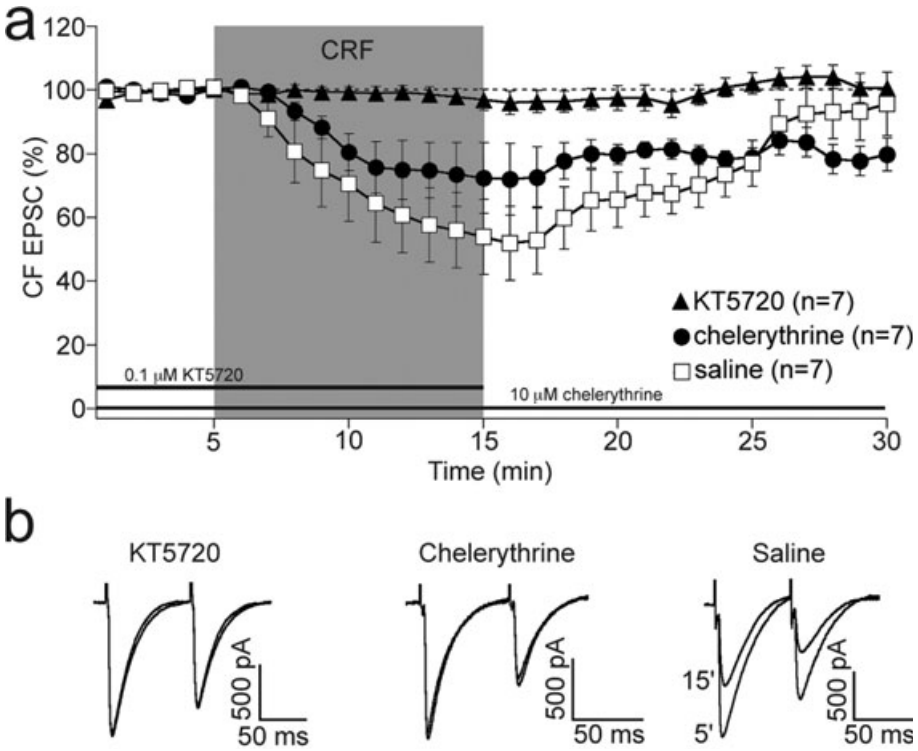

FIG. 4. CRF effect upon the climbing fibre-Purkinje cell EPSC is protein kinase mediated. (a) Inhibitors of PKC (chelerythrine) or PKA (KT5720) impaired the effect of CRF (1.0 μM) upon the CF-evoked Purkinje cell EPSC. (b) Example traces of EPSCs at minute 5 and 15. Whereas the PKC inhibitor reduced the CRF effect by only half on average, in several cases such as the example shown here the block was complete.

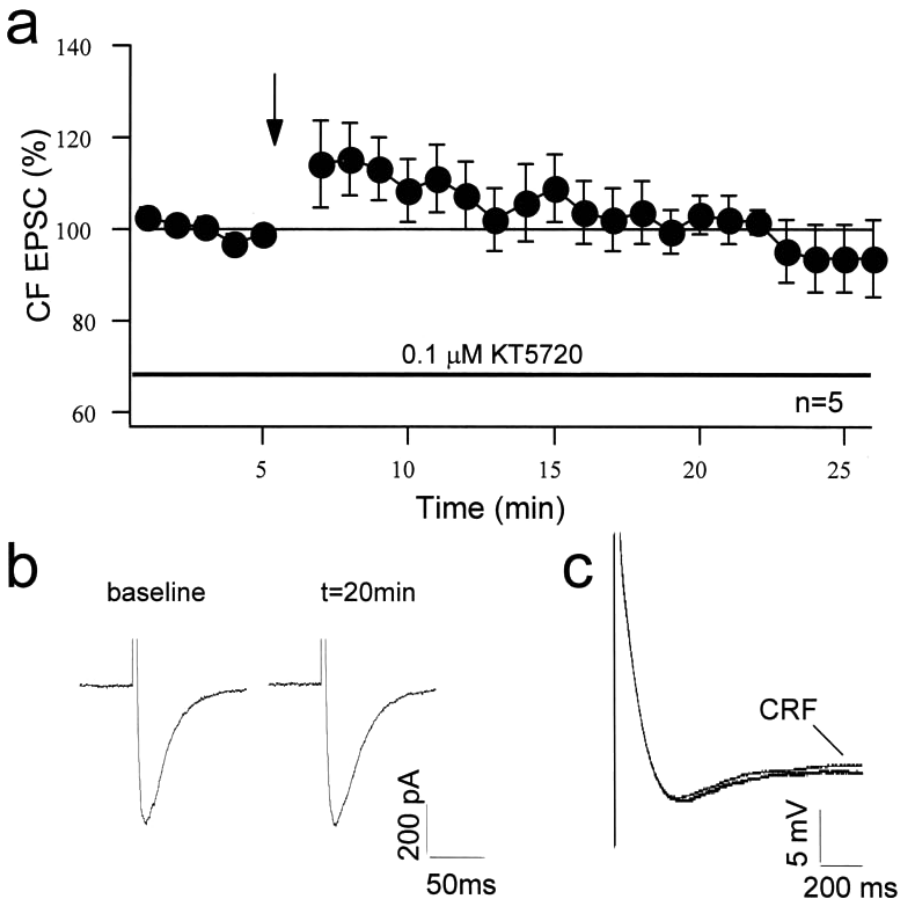

FIG. 5. PKA inhibition blocks CF-LTD induction as well as the CRF-mediated reduction of the AHP amplitude. (a) Bath application of the PKA inhibitor KT5720 (0.1 μM) impaired CF-LTD induction ($n = 5$). The arrow indicates the time point of CF tetanization. (b) Typcial CF-EPSCs before and after tetanization. (c) When KT5720 (0.1 μM) was bath-applied, CRF (1.0 μM) application did not cause a reduction in the amplitude of complex spike-associated AHPs ($n = 4$).

involvement of PKA, we applied the CF-LTD protocol (5 Hz, 30 s) in the presence of KT5720, a potent PKA inhibitor. KT5720 (0.1 μM) was applied to the bath throughout the recordings. On average, KT5720 blocked the induction of CF-LTD (103 ± 2.7%, $t = 20$ min, $n = 5$) when compared with baseline ($t = 5$ min, $n = 5$, $P > 0.05$; Fig. 5a) or to CF-LTD under saline conditions (84 ± 3.8%, $t = 20$ min, $n = 9$, $P < 0.05$; Fig. 3b). One of five cells in the KT5720 condition demonstrated > 10% reduction of the CF-EPSCs 20–25 min after CF tetanization, compared with seven of nine cells in the saline condition.

Finally, to test whether the CRF effect on the complex spike-associated AHP is also PKA-dependent, we recorded complex spikes and the subsequent AHPs when KT5720 (0.1 μM) was bath-applied. In contrast to the control saline bath condition (Fig. 1b), bath application of KT5720 blocked the CRF- (1.0 μM) mediated reduction of AHP amplitudes (Fig. 5c; $n = 4$).

Discussion

Here we show for the first time that CRF, a neuropeptide that is released from CF terminals under high activity conditions, regulates the amplitude of EPSCs and synaptic plasticity at the CF-PC synapse.

CRF regulates CF-PC synaptic efficacy

Endogenous CRF is supplied to the cerebellar cortex by both mossy fibres and CFs (Palkovits *et al.*, 1987; Van den Dungen *et al.*, 1988) and PCs express type 1 and type 2 CRF receptors (Bishop *et al.*, 2000; Swinny *et al.*, 2003; Gounko *et al.*, 2006). Here, we find that focally applied exogenous CRF suppresses the PC slow AHP following either somatic current injection or CF activation (Fig. 1). In addition, we find that exogenous CRF suppresses component 2 of the complex spike while component 1 remains unaffected. These results support and extend previous experiments in which CRF was bath-applied to PCs in cultures (Fox & Gruol, 1993) or cerebellar slices (Miyata *et al.*, 1999). They also reveal that three separate features of the PC complex spike are modified by the presence of CRF in exactly the same manner as they are by CF-LTD induction (Hansel & Linden, 2000; Schmolesky *et al.*, 2005). CRF might exert its influence on the complex spike through the same mechanism posited for CF-LTD: by reducing AMPA receptor-mediated currents. To test this prediction we bath-applied exogenous CRF and observed its effect upon the CF-EPSC. The application of CRF caused a dramatic, yet reversible, reduction in CF-EPSCs (Fig. 2) that was blocked by co-application of the CRF receptor antagonist astressin (Fig. 3). The application of astressin alone had no effect upon the CF-EPSC. Taken together, these results suggest three things. First, the basal endogenous concentration of CRF does not regulate CF-PC synaptic efficacy in the cerebellar slice. If it did we would expect the CRF-R antagonist to influence the CF-EPSC, which it did not. Second, moderate concentrations of CRF (such as the 1.0 μM applied here) are capable of temporarily reducing CF-PC synaptic efficacy by half. Thus, activity-driven release of higher concentrations of CRF could have a significant, if transient, impact upon glutamatergic transmission at the CF-PC synapse. Third, it

appears likely that the effects of CRF upon the complex spike are, in some part, the direct result of an attenuated CF-EPSC. This supposition is based upon three facts: (1) pharmacologically suppressing the CF-EPSC with NBQX causes impaired CF-evoked PC dendritic Ca^{2+} transients; (2) the complex spike AHP is Ca^{2+}-mediated; and (3) reduced CF-evoked Ca^{2+} transients are associated with an attenuation of the complex spike AHP and second component (Schmolesky *et al*., 2002, 2005; Weber *et al*., 2003). That said, CRF also impairs the slow AHP evoked by somatic current injection (Fig. 1; Fox & Gruol, 1993), a case in which glutamatergic receptors are not involved. Thus, as suggested by Miyata *et al*. (1999), CRF might also influence K^+ channels directly. Voltage-gated Ca^{2+} channels are not a likely target of CRF as Ca^{2+} transients evoked by somatic current injection are not affected by CRF application (Miyata *et al*., 1999).

Postsynaptic effects of CRF

A recent report by Liu *et al*. (2004) demonstrates that CRF may postsynaptically influence glutamatergic transmission in both the amygdala and lateral septum. Type 1 CRF receptors (CRF-R1s) in the cerebellum are expressed in the proximal PC dendrite across from PF terminals and in non-synaptic regions, but not across from CF terminals (Swinny *et al*., 2003). The absence of CRF-R1 expression in the PC dendritic region across from CF terminals is somewhat surprising given the prominent CRF labelling in these terminals. This absence might be explained by high rates of ligand–receptor coupling followed by CRF receptor internalization (Bloch *et al*., 1999; J. D. Swinny, personal communication). Alternatively, CF-supplied CRF could act on CRF-R1s via diffusion to adjacent PC dendritic or somatic regions. Type 2 CRF receptors (full length) are expressed in both the somata and proximal dendrites of PCs in postnatal day 15 and 30 rats (Gounko *et al*., 2006). Electron immunochemistry revealed that these receptors are not expressed in PC dendritic spines across from CF and PF terminals, but are localized to postsynaptic regions of PC dendritic shafts most likely related to CF terminals (Gounko *et al*., 2006). It is important to note that both CRF-R1s and R2s are expressed in the CF presynaptic terminal (Swinny *et al*., 2003; Gounko *et al*., 2006). Although we cannot exclude the possibility that CRF acts presynaptically to impair CF-PC synaptic efficacy, the fact that the CF-EPSC PPD ratio was unaffected by CRF application (Fig. 2) argues for a postsynaptic site of influence for the effects described herein. We speculate that presynaptic CRF binding on the CF terminal may serve an autoregulatory function, as has been described for other neuropeptides (Merighi, 2002).

Endogenous CRF receptor binding is required for the induction of CF-LTD

The current data clearly demonstrate that exogenous CRF is capable of influencing CF-evoked sub- and supra-threshold events. These effects were transient, indicating that a moderate rise in [CRF] is, by itself, insufficient to induce CF-LTD. However, attempts to induce CF-LTD in the presence of the CRF receptor antagonist astressin failed in 12 of 13 cases (Fig. 3). Thus, concomitant with increases in mGluR1 activation, dendritic $[Ca^{2+}]_i$ and PKC activity (Hansel & Linden, 2000), endogenous ligand at some undetermined concentration must bind CRF receptors for the induction of CF-LTD to occur. The required levels of endogenous ligand could be supplied through the basal release of CRF (mossy fibres or CFs), basal release of the CRF-related peptide urocortin (parallel fibres or CFs; Swinny *et al*., 2002) or, as we believe to be most likely, activity-driven release of CRF from CFs. Future studies will examine the combination of various signals (e.g. CRF-R activation and PC somatic depolarization; CRF-R activation, mGluR activation, and PC somatic depolarization, etc.) to assess minimal requirements and thresholds for converting transient CF EPSC depression to LTD.

Potential CRF signal transduction pathways

The action of CRF is exerted by binding to type 1 and/or type 2 CRF receptors. Multiple receptor splice variants exist and receptor binding may activate a wide range of coupled G-proteins (Grammatopoulos *et al*., 2001) and, thereby, transduction events. Reports on CRF action in diverse cell types indicate the ability of this peptide to activate the adenylyl cyclase/PKA and PKC pathways (Grammatopoulos *et al*., 2001; Blank *et al*., 2003). Studies have shown that both PF-LTD and CF-LTD induction require heightened PKC activity (Hansel & Linden, 2000; Hansel *et al*., 2001; Weber *et al*., 2003). It is clear that mGluR1 binding contributes to increased PKC activity. Logic suggests that basal rates of CRF-R binding might help set the PKC tone. In addition, elevated CRF-R binding during periods of high activity could act to raise PKC levels above threshold for synaptic modification. In support of these hypotheses, biochemical assays demonstrate that CRF application significantly increases PKC activity in cerebellar slices (Miyata *et al*., 1999). Consistent with this observation, we find that PKC inhibition substantially reduces the effect of CRF upon the CF-PC EPSC (Fig. 4).

Thus, the CRF-mediated acute and reversible reduction of CF-EPSC amplitudes on the one hand, and CF-LTD induction on the other hand (reflected in a persistent reduction of CF-EPSC amplitudes) share a dependence on PKC activity. This observation indicates that these processes are related by recruiting the same signalling cascades and suggests that CRF facilitates CF-LTD induction by activating PKC. The same argument can be applied to the participation of PKA: the activation of PKA is required for the CRF-mediated reduction of CF-EPSCs (Fig. 4) and complex spike-associated AHPs (Fig. 5). In addition, PKA activation appears crucial for CF-LTD induction (Fig. 5). This finding came as a surprise as perfusion of Purkinje cells with cAMP derivatives failed to induce PF-LTD (Ito & Karachot, 1992), which suggests that PKA might not be involved in PF-LTD induction. It remains to be determined what specific roles PKC and PKA, respectively, play in CF-LTD induction. Direct PKA effects on AMPA receptor function have not been reported so far in Purkinje cells. However, recent studies revealed that the AMPA-R phosphorylation underlying cerebellar LTD relies not only upon the activation of kinases, but also on the inhibition of phosphatases (Eto *et al*., 2002; Launey *et al*., 2004). Thus, CRF-R activation of PKA could lead to the activation of phosphatase inhibitor proteins and, thereby, cause a transient or persistent reduction in the CF-EPSC. In conclusion, activity at the CF-PC synapse plays a central role in cerebellar function by driving the complex spike, influencing simple spike throughput, elevating dendritic Ca^{2+} levels and acting as a necessary associative signal for heterosynaptic PF-LTD. Plasticity at the CF-PC synapse is therefore likely to influence ongoing cerebellar processing (Schmolesky *et al*., 2002, 2005), neuroprotection (Weber *et al*., 2003) and memory formation (Coesmans *et al*., 2004). This study shows, for the first time, that the neuropeptide CRF could influence these neural functions by transiently suppressing CF-PC efficacy or facilitating CF-LTD. Moreover, this study contributes to the characterization of

cellular mechanisms underlying CF-LTD induction by showing that CF-LTD is PKA-dependent.

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Abbreviations

ACSF, artificial cerebrospinal fluid; AHP, afterhyperpolarization; CF, climbing fibre; CRF, corticotropin-releasing factor; EPSC, excitatory postsynaptic current; LTD, long-term depression; LTP, long-term potentiation; mGluR, metabotropic glutamate receptor; PC, Purkinje cell; PF, parallel fibre; PKA, protein kinase A; PKC, protein kinase C; PPD, paired pulse depression.

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