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ERK1/2 ACTIVATION IN RAT VENTRAL TEGMENTAL AREA BY THE μ-OPIOID AGONIST FENTANYL: AN *IN VITRO* STUDY

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Abstract—Opioid receptors in the ventral tegmental area, predominantly the μ-opioid receptors, have been suggested to modulate reinforcement sensitivity for both opioid and non-opioid drugs of abuse. The present study was conducted to study signal transduction proteins, which may mediate the functioning of μ-opioid receptors in the neurons of the ventral tegmental area. Therefore, brain slices of the ventral tegmental area were exposed *in vitro* to the specific μ-opioid agonist fentanyl and immunohistochemically stained for four different activated proteins using phospho-specific antibodies. Fentanyl dose-dependently activated extracellular signal-regulated protein in brain slices of the ventral tegmental area. This activation was reversible with naloxone. Furthermore, naloxone itself also activated extracellular signal-regulated protein kinase. Under the present conditions fentanyl did not affect extracellular signal-regulated protein kinase 1 and 2, Stat and cyclic AMP-response element-binding protein activity. The direct activation of extracellular signal-regulated protein kinase in ventral tegmental area slices by the μ-opioid agonist fentanyl may suggest a role of extracellular signal-regulated protein kinase in reward processes.

Key words: immunohistochemistry, phospho-proteins, addiction.

Endogenous opioid systems have been implicated in reinforcement. Especially μ-opioid receptors in the ventral tegmental area (VTA) appear to be involved in both cocaine and ethanol reinforcement (Van Ree et al., 1999). For instance, self-administration studies in rats suggest that μ-opioid receptors in the VTA can modulate the initiation of cocaine self-administration. In both drug-naive rats and mice, treatment with opioid antagonists decreased cocaine intake (De Vry et al., 1989; Kuzmin et al., 1997). In fact, naltrexone caused a rightward shift in the dose-response curve for cocaine, indicating that cocaine is less reinforcing after opioid blockade. Furthermore, local injection of the opioid antagonist naltrexone into the VTA also reduced acquisition of cocaine self-administration, whereas injections of naltrexone in the caudate, amygdaloid or accumbens nuclei as well as in the prefrontal cortex did not affect cocaine self-administration (Ramsey et al., 1999). Injection of the specific μ-opioid receptor agonist DAMGO and the antagonist CTOP into the VTA also altered reinforcement processes for cocaine (Corrigall et al., 1999). Taken together these studies suggest that μ-opioid receptors in the VTA are involved in sensitivity to drugs of abuse.

The cloning of the opioid receptors has facilitated the investigation of signalling pathways involved in opioid receptor mediated functioning. The elucidation of signal transduction pathways coupled to the μ-opioid receptor in neurons in the ventral tegmental area may add further to the understanding of mechanisms underlying differences in sensitivity to drugs of abuse. From studies using transfected cell lines it appears that opioid receptors are coupled to multiple signal transduction pathways. Due to the availability of antibodies against different phospho-proteins it is now possible to investigate signal transduction pathways *in situ* (Reijmers et al., 2000). *In vivo* studies suggest a role of cyclic AMP (cAMP)-response-element binding protein (CREB), extracellular signal-regulated protein kinase (ERK) and ERK kinases 1 and 2 (MEK1/2) in opioid receptor mediated signalling (Berhow et al., 1996; Guitart et al., 1992; Lane-Ladd et al., 1997; Ortiz et al., 1995; Schulz and Höllt, 1998; Widnell et al., 1996). However, these studies used nonselective opioid agonists which were injected systemically, either acute or chronically. Most of these studies did not concern the VTA: only Berhow et al. (1996) described activation of ERK in the VTA after chronic but not acute systemic morphine treatment. In the present study, signal transduction proteins coupled to the μ-opioid receptor in brain slices of the VTA were investigated. The *in vitro* approach was chosen to ensure detection of acute and local effects in the VTA. After stimulation with the specific μ-opioid receptor agonist fentanyl immunoreactivity was determined for phosphorylated CREB, ERK, MEK1/2 and Stat. These proteins were chosen based on known signal transduction pathways and availability of phospho-specific antibodies.

EXPERIMENTAL PROCEDURES

Animals and tissue preparation

Male Wistar rats (200 g; GDL Utrecht University) were housed in pairs in Macrolon© type III cages with water and food pellets *ad libitum*; environmental conditions were controlled (22°C and 50% humidity; lights on at 7:00 h and lights off at 19:00 h). The experimental procedures were approved by the Ethical Committee for Animal Experiments of Utrecht University.

For the *in vitro* procedures rats were killed by decapitation and brains were quickly dissected and transferred to ice-cold

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Abbreviations: CREB, cyclic AMP-response-element-binding protein; ERK, extracellular signal-regulated protein; IR, immunoreactivity; MAPK, mitogen-activated protein kinase; MEK, extracellular signal-regulated protein (ERK) kinase; TTX, tetrodotoxin; VTA, ventral tegmental area.

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Krebs–Ringer solution (124 mM NaCl, 3.3 mM KCl, 1.2 mM KH_2PO_4, 1.3 mM $MgSO_4$, 10 mM glucose, 20 mM $NaHCO_3$, 2.5 mM $CaCl_2$). Midbrain tissue blocks were cut from approximately −4.8 mm to −7.3 mm posterior to bregma, according to the Rat Brain Atlas (Paxinos and Watson, 1998). The tissue was fixed on a specimen plate with cyanoacrylate glue and 2% agarose, and subsequently 500 μm coronal vibratome (Vibratome® Series 1000, Technical Products International Inc., St. Louis, USA) slices were cut in ice-cold Krebs–Ringer solution, oxygenated with 95%O_2/5%CO_2. As an example, one hemisphere of a slice is schematically depicted in Fig. 1 A. Of each animal two slices cut at the level of the VTA were included in the experiment, which were assigned to one treatment group and were considered one sample.

In vitro procedures

The 500-μm-thick VTA slices were allowed to rest for 1.5 h at room temperature in oxygenated Krebs–Ringer solution. Slices were then transferred to oxygenated 30°C Krebs–Ringer solution for another 30 min: 15-min acclimatisation followed by 15-min treatment.

Experiments

Experiment 1, screening. With phospho-specific antibodies the possible involvement of four intracellular signal transduction proteins in ventral tegmental μ-opioid receptor mediated signalling was studied. Herefore, rat VTA brain slices were stimulated with the μ-opioid receptor agonist fentanyl (Janssen Pharmaceutica B.V., Tilburg, The Netherlands) *in vitro* (n=4). The experimental procedures were validated using three control groups. *Fresh tissue* and *pre-incubated tissue*, i.e. tissue which was fixed after a total 2 h (1.5 h at room temperature and 30 min at 30°C) incubation in Krebs–Ringer without additives, were included to check for pre-incubation effects. Furthermore, to check for tissue viability, tissue was stimulated with *50 mM KCl* (KCl was added to the medium at t=105 min).

For the experimental groups the sodium channel blocker *tetrodotoxin* (TTX, 1 μM, Tocris, UK) was used to prevent indirect effects due to depolarization of target cells (TTX was added to the incubation medium at t=90 min). The three experimental groups were TTX alone, TTX+0.1 μM fentanyl and TTX+0.5 μM fentanyl (fentanyl was added to the medium at t=105 min). The 15-min incubation time with fentanyl was chosen because according to many studies the peak activity of most phospho-proteins lies within the range of 10-20 min. The fentanyl concentrations were chosen from a study on inositol (1,4,5)-triphosphate formation by fentanyl in SH-SY5Y neuroblastoma cells (Smart et al., 1994) and comparable studies on cell cultures which used either DAMGO or morphine (K_D in the range of that of fentanyl, Ai et al., 1999; Fukuda et al., 1996; Gutstein et al., 1997; Johnson et al., 1994; Li and Chang, 1996; Polakiewicz et al., 1998; Schmidt et al., 2000; Selley et al., 1997). After a total 2 h of incubation the slices were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) overnight. The slices were stored in 0.1% sodium-azide in 0.1 M Tris-buffered saline (TBS, pH 7.4) until further processing.

Experiment 2, ERK activation repeat experiment. To confirm the observed dose-dependent activation of ERK by acute stimulation with fentanyl the experiment was repeated with the same doses of fentanyl: *TTX* alone, *TTX + 0.1 μM fentanyl* and *TTX + 0.5 μM fentanyl* (n=6). Fresh tissue was not included because ERK activity was not different in pre-incubated tissue compared with fresh VTA slices in experiment 1. As an internal control, additional measurements were made in the substantia nigra reticularis, in which area moderate levels of μ-opioid receptors are expressed (Ding et al., 1996).

Experiment 3, specificity of ERK activation for the μ-opioid receptor. To check for specificity of the effects of fentanyl upon p-ERK immunoreactivity for the μ-opioid receptor the opioid antagonist naloxone was used. The concentration of naloxone was 5 μM; naloxone was added at t=100 min. Previous studies used either 1 or 10 μM naloxone to demonstrate opioid receptor involvement in responses to DAMGO or morphine (Ai et al., 1999; Fukuda et al., 1996; Gutstein et al., 1997; Polakiewicz et al., 1998; Schmidt et al., 2000; Smart et al., 1994). Based on these studies the intermediate dose of 5 μM naloxone was chosen to examine opioid receptor involvement in the fentanyl-induced activation of ERK in this study. One micromole of TTX-treated slices and tissue slices exposed to the combination of 1 μM TTX and 0.5 μM fentanyl were included, which had been incubated in presence or absence of naloxone (n=8).

Immunohistochemistry

Polyclonal rabbit antibodies against phosphorylated kinases and transcription factors were chosen from a broad range of signal transduction pathways. We used antibodies against phosphorylated ERK (=p42/p44 ERK) from Promega (Madison, WI, USA), MEK1/2 (p-MEK1/2; Ser217/221) and p-Stat (Tyr705) from New England Biolabs (Beverly, MA, USA), and p-CREB antibody from Upstate Biotechnology (Waltham, MA, USA). These antibodies were specific as checked on a Western blot loaded with VTA homogenate.

The fixed VTA slices were cut down to 40-μm thick vibratome slices and free-floating immunohistochemistry was performed. Slices (one in Experiment 1, two in Experiment 2) from two to three animals within one treatment group were processed within the same incubation chamber and a net-well system was used to ensure that incubation times were exactly the same for all groups. Slices were rinsed with TBS, preincubated with 5–10 mg/ml sodiumborohydride in TBS for 20 min rinsed with TBS, preincubated with 3% H_2O_2 in TBS and rinsed again. The slices were incubated in supermix (TBS with 0.5% Triton X-100 and 0.25% gelatine) containing a phospho-specific antibody (anti-p-ERK (1:3200), anti-p-MEK1/2 (1:800), anti-p-Stat (1:800) or anti-p-CREB antibody (1:2500)) for 1 h at room temperature followed by 48 h at 4°C whilst shaking on a rocking table. Slices were again rinsed with TBS and incubated with biotinylated goat anti-rabbit IgG (1:500; Vector, Burlingame, CA, USA) in supermix for 1 h at room temperature whilst shaking. After rinsing with TBS the slices were then incubated with avidin-biotin complex coupled to peroxidase (1:1000 Vectastain ABC; Vector) in supermix for 2 h at room temperature. Finally, the slices were rinsed with TBS and stained with 0.5 mg/ml diaminobenzidine (Sigma, Zwijndrecht, Netherlands) in TBS containing 0.2% nickelammoniumsulphate and 0.01% H_2O_2. The enzymatic reaction was stopped in TBS and the slices were mounted on gelatine-coated slides, dehydrated in graded ethanol, embedded and coverslipped.

Image analysis

Slices were examined and images were taken using a MCID image analyser (Interfocus, Suffolk, UK) coupled to a microscope (Carl Zeiss, B. V., Weesp, The Netherlands). For quantitative analysis of the immunoreactivity (IR) for the different phosphoproteins, performed with the same MCID system, we used images of the VTA (20× objective) at −5.80 mm posterior to bregma, according to the Rat Brain Atlas.

The staining pattern for p-CREB, p-MEK1/2 and p-Stat was punctate, hence proportional grain area was chosen for quantification of the IR for these proteins. The staining pattern for p-ERK was more diffuse and therefore optical density was used as the parameter for the quantification of p-ERK IR. Per animal average immunoreactivity was calculated from single measurements from

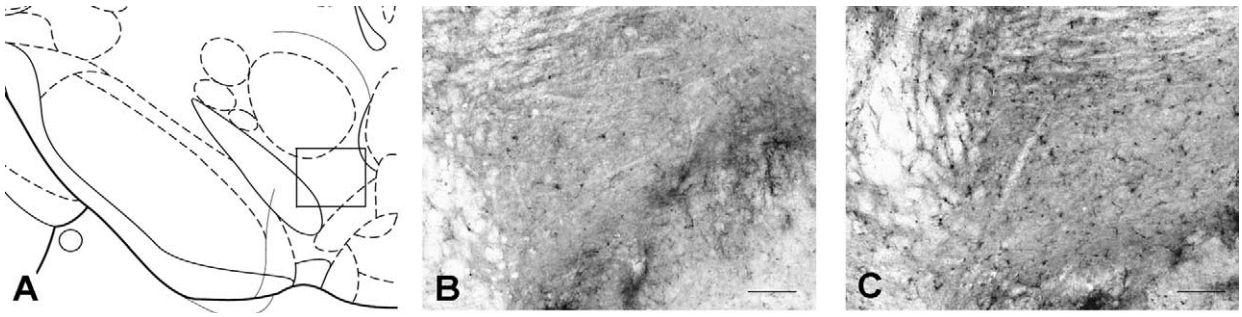

Fig. 1. Phospho-ERK immunoreactivity in VTA brain slices treated *in vitro* with fentanyl. Images were taken at −5.80 mM to bregma according to the Rat Brain Atlas (Paxinos and Watson, 1998) as schematically drawn in (A). Panel (B) shows p-ERK immunoreactivity for TTX-treated tissue and panel (C) shows a representative example of TTX+0.5 μM fentanyl-treated VTA slices. Calibration bar: 100 μm.

both hemispheres of either one (experiment 1) or two slices (experiments 2 and 3).

Statistical analysis

For statistical analysis of the data one- or two-way ANOVA (SPSS© 9.0 for Windows) was used, followed by post-hoc comparisons (Tukey test). Overall analysis compared TTX, TTX+0.1 μM fentanyl and TTX+0.5 μM fentanyl groups for experiments 1 and 2. Similar analysis was performed after combination of the data of the experiments 1 and 2, taking the factor experiment into account. For the naloxone experiment an overall analysis was performed for TTX, TTX + naloxone, TTX + fentanyl and TTX + fentanyl + naloxone. In addition, separate analyses examined the effects of fentanyl (TTX vs TTX+0.5 μM fentanyl, in absence of naloxone) and effects of naloxone itself. Data are represented as mean±S.E.M.; significance was accepted at $P<0.05$.

RESULTS

Validation of experimental procedures

The data for the control groups for the different experiments are summarised in Table 1. Compared with fresh tissue, p-MEK1/2, p-Stat and p-CREB immunoreactivity (IR) was reduced after pre-incubation (Table 1a). Fifty micromoles of KCl stimulation did not affect the immunoreactivity for p-MEK1/2, p-Stat and p-CREB. For p-ERK no difference between fresh and pre-incubated tissue was observed (Table 1b). In the repeat experiment only pre-incubated tissue was included. Exposure to 50 mM KCl increased p-ERK immunoreactivity. TTX did not significantly affect IR for p-MEK1/2, p-Stat, p-CREB and p-ERK as compared with pre-incubated tissue (compare Tables 1 and 2).

In vitro activation of signal transduction proteins by fentanyl in the ventral tegmental area

Out of four antibodies tested only phosphorylated ERK (p-ERK) showed a response to 15 min *in vitro* incubation with fentanyl, a specific μ-opioid receptor agonist. Microscopic examination of the VTA slices stained for p-ERK revealed differences in the density of the staining across groups (Fig. 1): p-ERK IR was more dense for the fentanyl-treated slices than for the TTX-treated control slices. Quantitative analysis of the slices, for which we used optical density as a parameter, confirmed the microscopic observations (Table 2). Fentanyl increased p-ERK immunoreactivity (IR) in *in vitro* treated VTA slices (overall effect: $F(2,11)=9.635$, $P<0.01$). Post-hoc analysis revealed significant differences between TTX and TTX+0.1 μM fentanyl ($P<0.05$) and TTX+0.5 μM fentanyl ($P<0.01$), thus the ERK activation by fentanyl was dose-dependent. No effects of fentanyl upon p-MEK1/2, p-Stat or p-CREB IR (proportional grain area) were observed (see Table 2). Western blot proved specificity of the antibody for phosphorylated ERK (p42/p44 ERK) as shown in Fig. 2.

ERK activation by fentanyl in VTA slices *in vitro*

To verify the finding that fentanyl activated ERK in the *in vitro* approach the experiment was repeated and similar results were found. Data for p-ERK IR from the two experiments revealed that there was an overall dose-dependent effect of fentanyl upon p-ERK IR: fentanyl increased p-ERK IR relative to TTX control slices ($F(2,30)=6.690$, $P<0.01$) (Fig. 3A). No interaction between treatment and experiment was present ($F(2,30)=0.365$, $P=0.698$). Post-hoc analysis revealed

Table 1. Validation of experimental procedures

A	p-MEK1/2	p-Stat	p-CREB
	Proportional grain area		
Fresh tissue	0.046±0.001	0.044±0.009	0.038±0.008
Pre-incubated tissue	0.015±0.002	0.015±0.006	0.019±0.008
50 mM KCl[a]	0.020±0.005	0.015±0.006	0.020±0.021

B	p-ERK optical density	
	Experiments 1 and 2	Naloxone experiment (3)
Fresh tissue	0.171±0.037[b]	–
Pre-incubated tissue	0.247±0.039	0.237±0.018
50 mM KCl[a]	0.338±0.029	–

(A) Summary of the data for the control groups for p-MEK1/2, p-Stat and p-CREB IR from experiment 1 ($n=4$). (B) Data of the control groups for p-ERK IR across the experiments: experiment 1 and 2 together ($n=10$) and the naloxone experiment ($n=8$).
Data are represented as mean±S.E.M.
[a] Pre-incubated tissue.
[b] Data only from experiment 1.

Table 2. Exposure of ventral tegmental area brain slices to fentanyl: effects upon signal transduction proteins (experiment 1)

	p-ERK	p-MEK1/2	p-Stat	p-CREB
	Optical density	Proportional grain area		
TTX	0.155±0.021	0.013±0.004	0.016±0.004	0.005±0.002
TTX+0.1 μM fentanyl	0.219±0.013[a]	0.022±0.009	0.024±0.003	0.010±0.003
TTX+0.5 μM fentanyl	0.246±0.017[b]	0.020±0.008	0.017±0.003	0.008±0.002

TTX was added during pre-incubation. The data are shown for p-ERK (optical density) and for p−MEK1/2, p-Stat and p-CREB (proportional grain area). Data are represented as mean±SEM.
Post-hoc Tukey tests: [a] vs. TTX tissue, $P<0.05$ and [b] vs. TTX tissue, $P<0.01$ ($n=4$).

significant differences in p-ERK IR between TTX and both 0.1 μM fentanyl and 0.5 μM fentanyl-treated tissue ($P<0.05$ and $P<0.01$, respectively). Additional measurements from the substantia nigra reticularis (SNR), which were included as internal controls, did not reveal activation of ERK by fentanyl. P-ERK IR in SNR: 0.124±0.031 for TTX, 0.130±0.015 for TTX+0.1 μM fentanyl and 0.116±0.028 for TTX+0.5 μM fentanyl.

Involvement of μ-opioid receptors in fentanyl-induced activation of ERK

Overall analysis revealed a significant interaction between naloxone and fentanyl treatment (treatment × naloxone $F(1,19)=4.588$, $P<0.05$) (Fig. 3b). Consistent with previous experiments we observed an increase in p-ERK IR in VTA slices after treatment with 0.5 μM fentanyl in the presence of TTX ($F(1,15)=9.180$, $P<0.01$). Naloxone itself increased p-ERK IR both in pre-incubated tissue without TTX treatment and in TTX-treated tissue. A two-way ANOVA revealed an effect of naloxone ($F(1,28)=5.309$, $P<0.05$).

DISCUSSION

In the present study we show activation of ERK, a member of the mitogen-activated protein kinase (MAPK) family, by the specific μ-opioid receptor agonist fentanyl in rat VTA brain slices. Furthermore, the opioid antagonist naloxone reversed the fentanyl-induced activation of ERK. Our data suggest that fentanyl activates ERK dose-dependently via an opioid-receptor mediated mechanism in VTA brain slices *in vitro*.

Out of four phospho-proteins only ERK showed a dose-dependent response to fentanyl treatment in VTA slices in an *in vitro* approach. Although opioid-mediated regulation of Stat has not been reported previously, effects upon ERK, MEK1/2 and CREB activity could be expected considering previous studies. For example, MEK1/2 activation by an opioid has been described previously, however only for the δ-opioid receptor agonist deltorphin (Hedin et al., 1999). Several studies reported CREB regulation by opioids. For example, Guitart et al. (1992) reported that

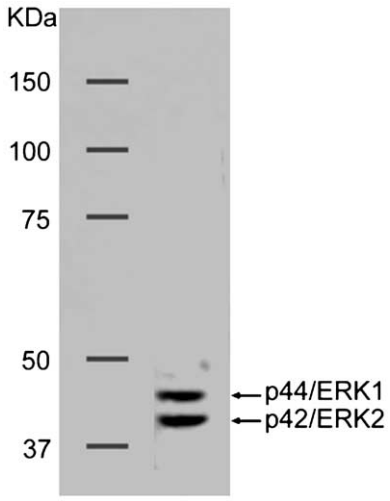

Fig. 2. Representative autoradiogram of phospho-ERK Western blot loaded with VTA homogenate. The detection of merely p42 and p44 ERK confirms the specificity of the antibody for phosphorylated ERK (=p42/p44 ERK).

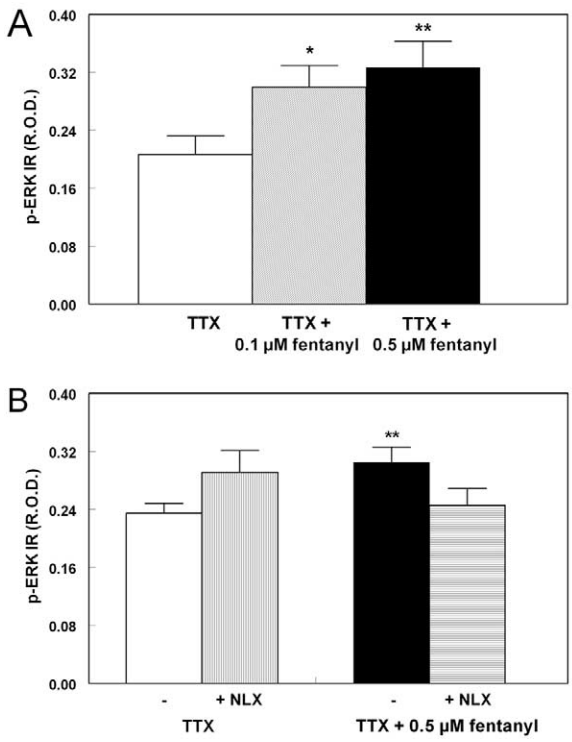

Fig. 3. ERK activation by fentanyl in VTA brain slices *in vitro*. Fentanyl increased p-ERK IR dose-dependently in rat VTA slices (A) ($n=10$). The activation of ERK by fentanyl is reversible with the opioid antagonist naloxone (B) ($n=8$). Data are represented as mean ± S.E.M. * vs. TTX, $P<0.05$; ** vs. TTX, $P<0.01$.

acute morphine decreased the state of phosphorylation of CREB (Guitart et al., 1992). Acute precipitation of opiate withdrawal increased the levels of phosphorylated CREB. Furthermore, chronic exposure to morphine increased levels of CREB in the locus coeruleus of the rat (Lane-Ladd et al., 1997). Widnell et al. (1996) showed decreased CREB immunoreactivity in the nucleus accumbens after chronic but not acute morphine. The lack of opioid-mediated regulation of MEK1/2 and CREB in the present study may be explained by the use of different opioid agonists. CREB and MEK1/2 activation has only been reported for the relatively non-specific opioid agonist morphine and for the δ-opioid receptor agonist deltorphin, respectively. Therefore, opioid-induced activation of CREB and MEK may be mediated by δ- and κ- but not by μ-opioid receptors. Furthermore, different experimental conditions, e.g. treatment time and the brain region studied may explain the lack of MEK1/2 or CREB activation in the present study. For example, MEK1/2 activation by deltorphin peaked at 5-min treatment time and reached basal levels after 10 min (Hedin et al., 1999), suggesting that after 15-min exposure as was done in the present study MEK1/2 phosphorylation levels may have returned to basal levels. Thus although no effects upon MEK1/2, CREB or Stat phosphorylation state were observed, the possibility of their involvement in opioid-mediated signalling cannot be ruled out.

In transfected cell lines, ERK activation by opioid agonists has been shown previously (Ai et al., 1999; Belcheva et al., 1998; Fukuda et al., 1996; Gutstein et al., 1997; Li and Chang, 1996; Polakiewicz et al., 1998; Schmidt et al., 2000). *In vivo* studies on opioid-mediated ERK activation have mostly dealt with chronic effects of systemic morphine. Moreover, results of these studies were controversial: chronic exposure to morphine decreased ERK activity in one study (Schulz and Höllt, 1998), whereas the same treatment, but not acute morphine administration increased ERK activity in other studies (Berhow et al., 1996). Berhow et al.,. found increased ERK activity after chronic morphine in the VTA and hence their results may agree with the present findings obtained in brain slices after *in vitro* exposure.

ERK activation induced by an acute stimulation with a specific μ-opioid agonist in a physiologically relevant system, as the VTA brain slices used for the present study has not been shown previously. Further, the *in vitro* approach, the use of the sodium channel blocker TTX, preventing depolarisations, and naloxone blockade ensured the detection of merely direct cellular μ-opioid receptor mediated effects. Our findings indicate a possible role of ERK in the acute effects of μ-opioid agonists in the VTA. As such ERK may have a role in reward processes. In fact, a role of the MAPKs, of which ERK is a subtype, in cocaine responsiveness has been suggested previously. Treatment with a MEK inhibitor before cocaine reduced cocaine-induced hyperlocomotion (Valjent et al., 2000) and blocked sensitisation to the locomotor activating effects of cocaine (Pierce et al., 1999). Recently, Valjent et al. (2001) demonstrated ERK activation in the striatum and nucleus accumbens by Δ9-tetrahydrocannabinol (THC). Furthermore, inhibition of ERK by the MEK inhibitor SL327 impaired THC induced place preference. With regard to opioid reward, involvement of ERK has not been studied so far. It has been shown that MAPK is involved in μ-opioid receptor desensitisation: a feedback signal emanating from the MAPK pathway appears to be required for μ-opioid receptor desensitisation, although internalisation is not required for MAPK activation by opioids (Kramer and Simon, 2000; Polakiewicz et al., 1998).

Because ERK activation by fentanyl was dose-dependent, an *in vitro* approach as described here may further be applicable to monitor changes in sensitivity of the μ-opioid receptor system in, for example, animal models for altered sensitivity to reinforcing effects of drugs.

Interestingly, naloxone activated ERK in VTA slices for both preincubation and TTX conditions. Naloxone did block the effects of fentanyl upon ERK phosphorylation, thusly acting as an antagonist in the presence of fentanyl as expected. Our data suggest that naloxone may act as a partial agonist in absence of fentanyl with antagonistic properties in presence of fentanyl. In support of non-classical behaviour of this opioid antagonist, partial agonist actions as well as inverse agonist properties have been suggested for naloxone previously in transfected cell lines (Fukuda et al., 1998; Wang et al., 1999). Further, Cruz et al. (1996) showed inverse agonist activity of naloxone in guinea-pig ilea preparations. However, studies which used μ-opioid receptor transfected cell lines do not support the present findings: naloxone blocked opioid-induced effects upon ERK activity, but when administered alone naloxone did not affect basal ERK activity in transfected CHO-K1 cells (Ai et al., 1999). Because there are no indications of partial agonist-like properties of naloxone in ERK-activation from studies using cell lines, we may speculate that cell-specific properties of receptor activation or participation of other receptor systems in the VTA may account for the naloxone-induced activation of ERK in the present study.

In conclusion, we show that the specific μ-opioid receptor agonist fentanyl induced ERK activation in a dose-dependent manner in rat VTA brain slices. Assuming a significant role of VTA μ-opioid receptors in reward processes (Van Ree et al., 1999) the signal transduction pathways involving ERK may be involved in the cellular mediation of reward as supported by cocaine sensitisation and THC place preference studies (Pierce et al., 1999; Valjent et al., 2001). The direct and dose-dependent activation of ERK may further provide a tool to test opioid efficacy, possibly in animal models relevant for addiction proneness. Future research on the intracellular mechanisms coupled to μ-opioid receptor activation in the VTA may provide more insight in the mechanisms underlying individual proneness to addiction and hence may contribute to the prevention of drug dependence.

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