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Biochemical Pharmacology 70 (2005) 762–769
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

The effects of desmethylimipramine on cyclic AMP-stimulated gene transcription in a model cell system

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Received 24 March 2005; accepted 7 June 2005

Abstract

The present study utilised an in vitro cell model of the cAMP signalling pathway to investigate the actions of desipramine (DMI) and other psychoactive agents on cAMP-driven gene transcription. The model comprised CHOβ_2 SPAP cells; Chinese hamster ovary cells expressing human β_2 adrenoceptors and a secreted placental alkaline phosphatase (SPAP) reporter gene with multiple cAMP response elements (CREs) in its promoter region. SPAP assays showed DMI to inhibit isoprenaline or forskolin-enhanced gene transcription in a time and concentration-dependent manner ($IC_{50} = 16.6 \pm 2.0$ μM after 18 h). This effect of DMI was not dependent upon activity at the levels of the β_2 receptor, cAMP accumulation or phosphorylation of the transcription factor, cAMP response element binding protein (CREB). The inhibitory effects were maintained in the presence of DMI for at least 3 weeks and were mimicked by exposure to norfluoxetine (the major metabolite of fluoxetine; $IC_{50} = 7.2 \pm 1.8$ μM) and the neuroleptics, chlorpromazine and clozapine, all at a concentration of 10 μM. Amphetamine (10 μM, 18 h) enhanced SPAP gene transcription. Ca^{2+} imaging experiments ruled out an inhibitory effect of DMI on Ca^{2+} influx as concluded by previous studies.

The results suggest a molecular target for DMI that lies downstream of CREB phosphorylation. Whether the inhibitory action of DMI is common to naturally expressed CRE-driven genes involved in adaptive responses to antidepressants in vivo remains to be determined.

Keywords: Antidepressant; Cyclic AMP; CHO cell; CREB

1. Introduction

Despite nearly 50 years of research, a common mechanism of action underlying the therapeutic actions of antidepressant drugs is still to be described. The short-term effects of the majority of antidepressants are widely assumed to be mediated by enhancing the synaptic availability of monoamine neurotransmitters, particularly noradrenaline and serotonin (5HT), via blockade of their reuptake or metabolism. This does not, however, explain the antidepressant properties of drugs such as tianeptine that *increase* 5HT uptake [1]. The prolonged delay before clinical benefit becomes apparent implies that acute actions trigger adaptive responses in the brain that reverse neuronal malfunctions responsible for depression [2]. The changes in intracellular signalling pathways and gene transcription that produce these adaptations are, however, incompletely understood [3].

Extensive evidence points to alterations in the cAMP signal transduction system [4–11] being strongly associated with antidepressant treatment. However, due to the lack of a wholly representative animal model of depression and the complexity of the neural networks affected by antidepressants in vivo it is difficult to identify relevant genes whose expression is altered as a result of such signalling changes.

Various reductionist approaches have been made to the problem using relatively simple in vitro cell models and observations of antidepressant-induced changes in signalling events in non-neuronal cells lacking monoamine transporters suggest that alternative acute targets exist (e.g. [12]). For example, Schwaninger et al. [13] proposed that antidepressants exert their inhibitory actions on cyclic AMP response element (CRE)-mediated gene transcription by blocking calcium influx into cells through voltage-dependent calcium channels (VDCC) in HIT and PC12 cells, preventing phosphorylation and activation of the transcription factor CREB (cyclic AMP response element binding protein). To explore this hypothesis, in the present

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doi:10.1016/j.bcp.2005.06.012

study, we have used a non-excitable Chinese Hamster Ovary (CHO) cell line stably transfected with the human β_2-adrenoceptor and a SPAP (secreted placental alkaline phosphatase) reporter gene [14], effectively producing an in vitro model of the cAMP signalling pathway.

The effects of desipramine (DMI) and other psychoactive agents on cAMP-driven gene transcription in these CHOβ_2 SPAP cells indicate that these drugs can modify gene transcription in the absence of effects on monoamine transport and metabolism or on voltage-dependent ion channels. The precise locus of action could not be identified but appears to be downstream of cyclic AMP response element binding protein (CREB) phosphorylation.

2. Materials and methods

2.1. Materials

Cell culture reagents were from Sigma Chemicals (Poole, Dorset, UK) except foetal calf serum (FCS) which was from PAA Laboratories (Teddington, Middlesex, UK). [^{3}H]-adenine and [^{14}C]-cAMP were obtained from Amersham International (Buckinghamshire, UK). ICI 118551 was from Tocris Cookson (Avonmouth, Bristol, UK). Western blotting reagents were supplied by BIORAD. Primary antibodies (anti-CREB and anti-phospho-CREB) were supplied by Cell Signalling technologies (New England Biolabs), secondary antibody (goat-antirabbit HRP-conjugated) was supplied by DAKO, UK. Emulsifier Scintillator Plus (scintillation fluid) were from Packard Biosciences (Perkin-Elmer, UK). All other reagents were supplied by Sigma Chemicals (Poole, Dorset, UK) unless otherwise stated.

2.2. Cell culture

Chinese hamster ovary (CHO) cells stably transfected with the human β_2-adrenoceptor (at an initial expression level of 310 fmol/mg protein) and a secreted placental alkaline phosphatase (SPAP) reporter gene (CHOβ_2 SPAP) [14], were grown in Dulbecco's modified Eagles medium/nutrient mix F12 (1:1) supplemented with 2 mM L-glutamine and 10% foetal calf serum (FCS) at 37 °C in an atmosphere of 5% CO_2:95% air. The SPAP gene is transcriptionally controlled by a promoter region containing six cyclic AMP response elements (CREs). Thus, any increase in intracellular cyclic AMP should provoke the appearance of SPAP in the extracellular medium.

2.3. Measurement of SPAP

Assays were performed on confluent cells grown in 24-well cluster dishes according to the method described by Cullen and Malim [15] and McDonnell et al. [14]. Briefly, cells were grown to confluency on 24-well cluster dishes before being serum starved in serum free Dulbecco's modified Eagles medium/nutrient mix F12 (1:1) supplemented with 2 mM L-glutamine only (sfm), for 24 h prior to assaying. The medium was aspirated and replaced with 1 ml per well fresh sfm. Agonists (isoprenaline 1 μM or forskolin 10 μM) were added to relevant wells and dishes were replaced in the incubator for 5 h. Medium was aspirated from all wells and replaced with 300 μl per well fresh sfm. Dishes were replaced in the incubator for a further 1 h. Twenty microlitres of samples from each well were transferred into 96-well cluster dishes which were then either frozen at −20 °C until required, or heated at 65 °C for 30 min (inactivates endogenous alkaline phosphatases). Two hundred microlitres per well *p*-nitrophenol phosphate (PNPP) reaction substrate was added before incubating dishes for a final hour at 37 °C. Hydrolysis of PNPP by SPAP was measured at 405 nm and data were converted to SPAP concentration in mU ml^{-1} using the equation shown below:

$$[\text{SPAP}]\,(\text{mU ml}^{-1}) = \frac{A}{t \times 18.5 \times V}$$

where A is the measured optical density at 405 nm, t is time with substrate (60 min) and V is the volume of sample.

2.4. cAMP accumulation

Cyclic AMP assays were conducted according to the single column separation method reported by Alvarez and Daniels [16] using cells grown to confluency on 24-well cluster dishes. Where required for 18 h pre-incubation, 20 μM DMI was applied to the cells at the same time as [^{3}H]-adenine (1 μCi per well) for overnight labelling.

Experiments were performed in Kreb's Henseleit buffer (KHB) warmed to 37 °C. Medium was aspirated from all wells and cells were washed with 1 ml per well KHB, before being replaced with 500 μl per well KHB containing 10 μM rolipram. Plates were incubated for 15 min at 37 °C. DMI and either isoprenaline or forskolin were added to wells in 5 μl additions from 100× stock solutions and plates were incubated fro a further 15 min at 37 °C before adding 50 μl 4.4 M HCl to stop reactions and permeabilise cell membranes. Plates were refrigerated for 30 min at 4 °C to allow for complete permeabilisation and column preparation (600 μg dry acid alumina per column). Fifty microlitres of supernatant from each well was transferred into 5 ml scintillation vials with 3.5 ml scintillation fluid before placing in a refrigerated scintillation counter and counting for total ^{3}H. Fifty microlitres of [^{14}C]-cAMP standard was then added to each well and plates were gently swirled to mix well contents. Well contents were then transferred onto individual, pre-prepared dry acid alumina columns. Columns were washed with 5 ml 5 mM HCl, then 1 ml 0.1 M ammonium acetate, discarding all eluate. Columns were then placed over 20 ml scintillation vials and washed with 3.5 ml 0.1 M ammonium acetate, collecting all eluate.

10 ml of scintillation fluid was added to each vial before placing in a refrigerated liquid scintillation counter and counting for ^{3}H and ^{14}C.

[^{3}H]-cAMP production was expressed as a percentage of [^{3}H]-adenine incorporation, assumed to be equivalent to total ^{3}H.

2.5. Western blotting

Western blotting was carried out according to manufacturer's instructions using the following antibodies: primary antibodies, phospho-CREB (ser 133) (polyclonal) and CREB antibody (polyclonal) were supplied by Cell Signalling Technology (New England Biolabs, UK). The secondary antibody, goat-antirabbit horseradish peroxidase (HRP)-conjugated, was supplied by DAKO, UK.

Briefly, 10% SDS-PAGE gels were prepared for use with the BIORAD Mini Protean III kit. On each gel lane 2 contained 10 μl BIORAD Kaleidoscope markers, lanes 4–9 contained samples and lanes 1, 3 and 10 were empty. Gels were run for 5–10 min at 100 V, *V* constant (until the markers started to separate), then at 200 V, *V* constant for ~45 min or until the dye front ran off the gel. Gels were transferred on to nitrocellulose membranes, again according to manufacturer's instructions, 100 V for 1 h. Membranes were blocked in blocking buffer (5% (w/v) Marvel, non-fat dried milk powder, in Tris-buffered saline, containing 0.1% Tween-20 [TBS-T]), for 1 h at room temperature. Membranes were then washed in TBS-T for 30 min before incubating with primary antibody (1:2000 in blocking buffer) overnight at 4 °C, with gentle shaking. Primary antibody solution was removed and membranes washed for 30 min in TBS-T, at room temperature, before incubating with secondary antibody (1:2000 in blocking buffer) for 1 h at room temperature, again with gentle shaking. Secondary antibody solution was discarded and membranes washed for a final time in TBS-T for 30 min, at room temperature. Washed membranes were exposed to ECL™ detection solution for 1 min before being quickly blotted dry on filter paper and wrapped in SaranWrap™. Wrapped membranes were placed in an A4 size X-ray cassette and exposed to Hyperfilm ECL™ autoradiography film for up to 15 min. Films were developed using an AGFA autodeveloper.

2.6. Statistical analysis

Statistical analysis of the data was performed by Student's *t*-test, with a $p < 0.05$ indicating a significant difference. Concentration–response curve fitting was performed by non-linear regression using the GraphPad Prism computer program (GraphPad, San Diego, CA, USA). Where used, one-way analysis of variance (ANOVA) on results with a significance of $p < 0.05$, or better, was performed using the SF ANOVA computer program.

Cyclic AMP accumulation is expressed as the percentage of the total incorporated ^{3}H-labelled products that are converted to [^{3}H]-cAMP.

3. Results

The β_2-adrenoceptor (β_2-AR) agonist isoprenaline increased extracellular SPAP in a concentration-dependent manner (EC_{50} = 10.7 ± 2.2 nM; Fig. 1). In order to verify that the isoprenaline response was β_2-AR-mediated, the experiment was repeated in the presence of the competitive β_2-AR antagonist ICI 118551 (8 nM). The calculated K_D for ICI was 0.9 nM (data not shown), identical to that reported for the same cell line by Baker et al. [17].

Cells exposed to DMI for 18 h (Fig. 1) revealed a significant reduction in maximal isoprenaline-stimulated SPAP production (E_{max} from 0.07 ± 0.01 to 0.02 ± 0.0003 mU/ml; $p < 0.001$, $n = 13$) and a small, but significant, rightward shift in the EC_{50} of the isoprenaline concentration–response curve (from 17.8 ± 5.1 to 36.3 ± 5.4 nM; $p < 0.05$, $n = 13$).

The effects of 18 h DMI exposure were concentration-related, showing an inhibition of isoprenaline-stimulated SPAP production with an IC_{50} value for DMI of 16.6 ± 2.0 μM ($n = 4$; Fig. 2A). The effect of DMI after 18 h was not specific to isoprenaline stimulation since the SPAP response to the adenylyl cyclase activator forskolin (10 μM) was also inhibited with an IC_{50} of 29.2 ± 8.9 μM ($n = 3$; Fig. 2B).

Effects of DMI were also evident, although less potent, after an exposure of only 1 h,with the SPAP response being completely inhibited at a concentration of 300 μM with IC_{50} values of 0.6 ± 0.2 mM ($n = 3$) for isoprenaline stimulation and 0.2 ± 0.03 mM ($n = 3$) for forskolin stimulation (Fig. 3). There was no observed change in gene transcription when DMI was added simultaneously (i.e. no pre-incubation) with either isoprenaline or forskolin (data not shown).

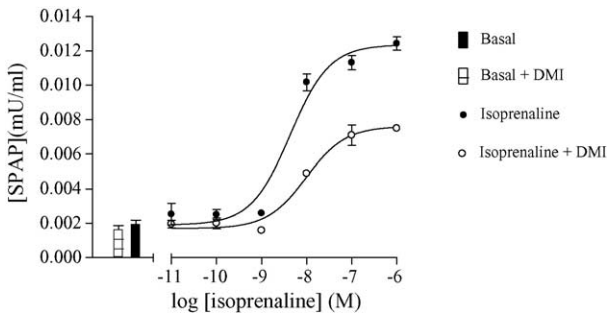

Fig. 1. Concentration–response curves to isoprenaline stimulation in the absence and presence of 20 μM DMI. Cells were preincubated with DMI for 18 h prior to isoprenaline stimulation. Data points are means ± S.E.M. of triplicates in a single representative experiment. This experiment was repeated a total of 13 times. Statistical analysis was done by unpaired Student's *t*-test.

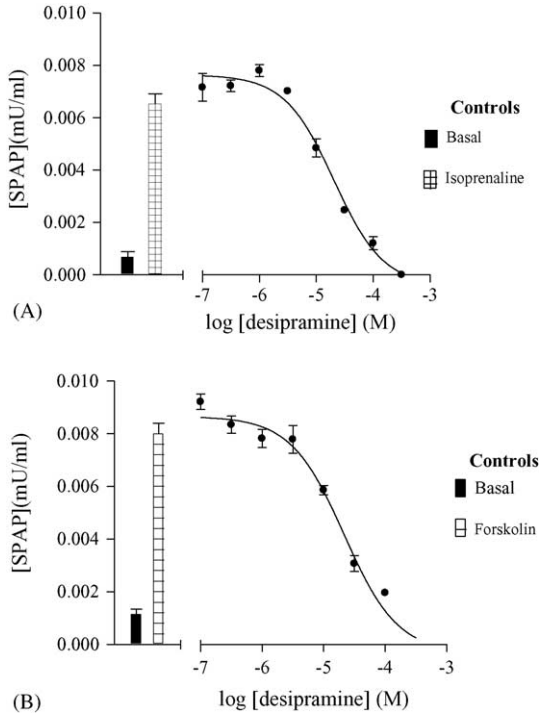

Fig. 2. SPAP responses to 1 μM isoprenaline stimulation (A) and 10 μM forskolin stimulation (B) in CHOβ_2 SPAP cells, after 18 h pre-incubation with DMI. IC_{50} values for DMI shown in text. Values are means ± S.E.M. of triplicate determinations in single representative experiments ($n = 4$).

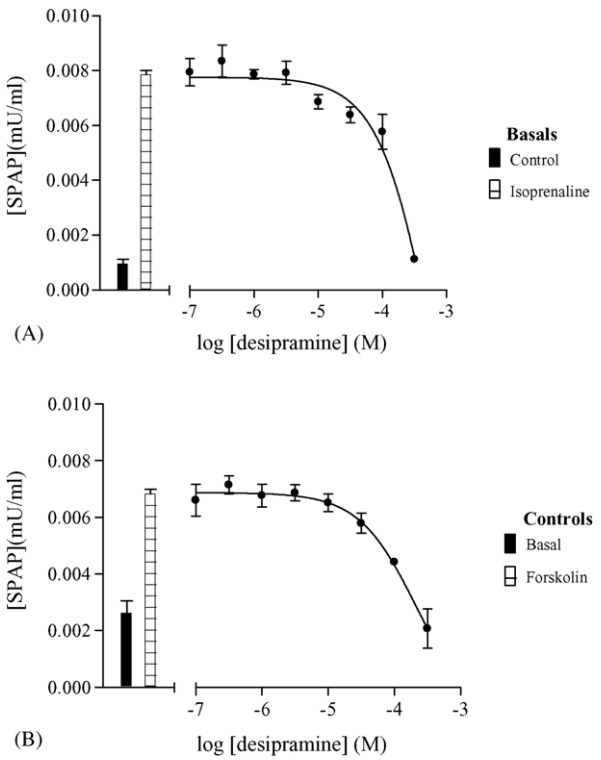

Fig. 3. SPAP responses to 1 μM isoprenaline stimulation (A) and 10 μM forskolin stimulation (B) after 1 h pre-incubation with increasing concentrations of DMI. IC_{50} values for DMI shown in text. Values are means ± S.E.M. of triplicate determinations in single representative experiments ($n = 4$).

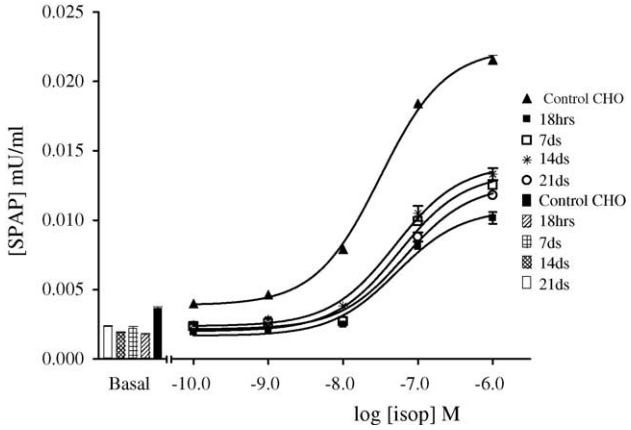

Fig. 4. SPAP responses to isoprenaline stimulation after chronic treatment with 20 μM DMI in CHOβ_2 SPAP cells. Data points are means ± S.E.M. of triplicates in single representative experiment, repeated on two further occasions ($n = 3$).

In order to study the longer term effects of antidepressants on CREB/CRE-mediated gene transcription CHOβ_2 cells were cultured for 18 h, 7, 14 or 21 days in the presence of 20 μM DMI prior to challenging with increasing concentrations of isoprenaline with subsequent measurement of the SPAP response (Fig. 4). There were no significant differences between any basal values or between the IC_{50}s of any of the curves as determined by one-way analysis of variance followed by Newman–Keuls post hoc test ($p > 0.05$). The reduction in isoprenaline E_{max} seen after 18 h exposure to DMI was maintained after 7, 14 and 21 days although there was a slightly reduced effect of DMI at these time points compared to 18 h exposure (not significantly different, $p > 0.05$; Newman–Keuls post hoc test).

To determine the effects of a different class of antidepressant drug, norfluoxetine (NFLX), the active metabolite of the selective serotonin reuptake inhibitor (SSRI) fluoxetine was employed. NFLX was used in preference to the parent compound as we were unsure of the capacity of CHO cells to metabolise fluoxetine. As with DMI, NFLX treatment of cells for 18 h resulted in a concentration-

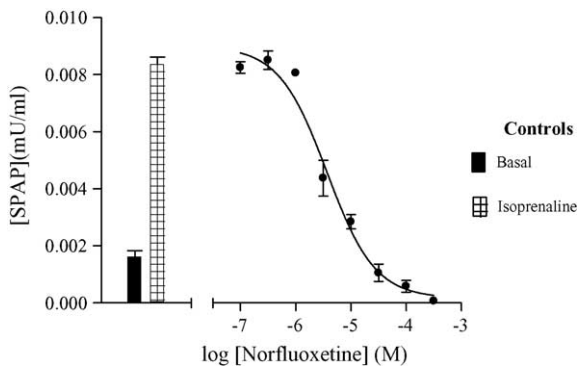

Fig. 5. SPAP response to 1 μM isoprenaline, after 18 h pre-incubation with increasing concentrations of norfluoxetine. Data points are means ± S.E.M. of triplicates within a single representative experiment ($n = 3$).

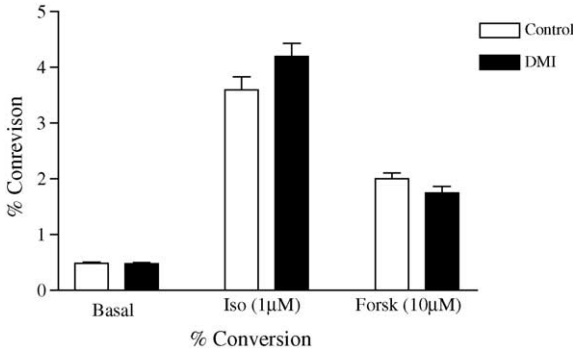

Fig. 6. cAMP accumulation in CHOβ_2 SPAP cells after 18 h incubation in the absence of presence of 20 μM DMI and subsequent stimulation with isoprenaline (Iso) or forskolin (Forsk). Data shown are the means from triplicates within three separate experiments (i.e. each bar is the mean of nine values). Control and DMI treatments were compared using an unpaired Student's *t*-test; there were no significant differences ($p > 0.05$).

related inhibition of SPAP gene transcription (IC_{50} = 7.2 ± 1.8 μM (n = 3); Fig. 5).

To determine whether the inhibition of gene transcription (observed with both DMI and NFLX) was specific to antidepressant agents, the effects of other psychoactive drugs on the CHOβ_2 SPAP model were investigated. The various psychoactive compounds listed in Table 1 were pre-incubated with the cells for 18 h before stimulating with 1 μM isoprenaline. Significant inhibitions of isoprenaline-stimulated SPAP production were observed after treatment with 20 μM DMI ($p < 0.01$), 10 μM clozapine ($p < 0.05$) and 10 μM chlorpromazine ($p < 0.01$). Exposure to 10 μM amphetamine significantly potentiated ($p < 0.01$) isoprenaline-stimulated SPAP production.

As with isoprenaline-stimulated SPAP production, 20 μM DMI, 10 μM clozapine and 10 μM chlorpromazine produced significant inhibitions of forskolin-stimulated SPAP production ($p < 0.001$, $p < 0.01$ and $p < 0.001$, respectively). 10 μM of amphetamine, once again, induced a significant increase in SPAP production ($p < 0.001$).

Table 1
Psychoactive drugs were preincubated with cells for 18 h prior to 1 μM isoprenaline stimulation

DRUG	% SPAP response to 1 μM isoprenaline
DMI (20 μM)	33.80 ± 3.04**
Cocaine (10 μM)	139.73 ± 12.72
Morphine (10 μM)	132.52 ± 12.06
Clozapine (10 μM)	67.04 ± 6.10*
Amphetamine (10 μM)	171.23 ± 15.58**
Diazepam (10 μM)	155.29 ± 14.13
Chlorpromazine (10 μM)	27.99 ± 2.55**
Lithium (2 mM)	72.95 ± 6.64
Sulpiride (10 μM)	88.64 ± 8.07
Haloperidol (10 μM)	114.68 ± 10.44

Values shown are means ± S.E.M. (n = 3–6). Statistical analysis was done by Newman–Keuls following a one-way analysis of variance, to determine the significance of inhibition/potentiation of isoprenaline-stimulated SPAP production (*$p < 0.05$, **$p < 0.01$). One micromolar of isoprenaline-stimulated SPAP was measured in each experiment. The SPAP response to 1 μM isoprenaline was 9.1 ± 1.1 fold control (i.e. that absence of isoprenaline). None of the drugs had a significant effect on SPAP production in the absence of isoprenaline.

CREB can be phosphorylated (and thereby activated) by increased intracellular Ca^{2+} acting via calcium calmodulin-dependent kinase (CaMK) [18]. Since acute application, in vitro, of several classes of antidepressant drugs has been reported to inhibit Ca^{2+} signalling [13,19–22] it could be suggested that the effects of the antidepressants on SPAP production are due to inhibition of Ca^{2+} signalling. This was tested by investigating the potential of isoprenaline to increase intracellular Ca^{2+} ($[Ca^{2+}]_i$) in CHOβ_2 SPAP cells loaded with FURA2-AM. Epi-fluorescent images of the cells were recorded at an emission wavelength of 510 nm after sequential excitation at 340 and 380 nm. 100 μM UTP or 10 μM ionomycin both produced robust increases in ($[Ca^{2+}]_i$) but 1 μM isoprenaline produced no response (data not shown) suggesting that the SPAP response to isoprenaline was indeed cAMP-mediated and Ca^{2+}-independent and that the antidepressant-induced reductions in expression must be unrelated to changes in $[Ca^{2+}]_i$.

The possibility of the antidepressant effect being mediated by changes in cAMP formation or metabolism was investigated by measuring ^{3}[H]-cAMP accumulation in CHOβ_2 SPAP cells pre-labelled with ^{3}[H]-adenine. As shown in Fig. 6, pre-incubation with 20 μM DMI for 18 h had no effect on the responses to a maximally effective concentration of isoprenaline (1 μM, previously determined, concentration–response data not shown) or to forskolin (10 μM).

Interaction with the activation, via phosphorylation, of CREB is another possibility for the antidepressant-induced inhibition of SPAP gene expression. Fig. 7 shows Western blots of samples prepared from CHOβ_2 SPAP cells treated for 1 h or 18 h with 20 μM DMI, prior to incubation with isoprenaline, using antibodies against CREB and CREB phosphorylated at serine 131. Analysis by densitometry revealed no changes in the expression of CREB or phospho-CREB following DMI exposure.

4. Discussion

The data presented clearly show that exposure of CHOβ_2 SPAP cells to the antidepressant drug DMI caused an inhibition of cyclic AMP-driven SPAP gene transcription from as early as 1 h after the start of incubation. The response was concentration-dependent and the potency of DMI increased with time of exposure, with the IC_{50} value changing from 600 μM at 1 h to 17 μM after 18 h. This concentration range is comparable with levels of tricyclic antidepressants detected in brain tissue after chronic treatment and might, therefore, have some relevance to the drug's therapeutic action [13].

The effect of the drug is likely to be due to changes in transcription of the SPAP gene rather than on export of the

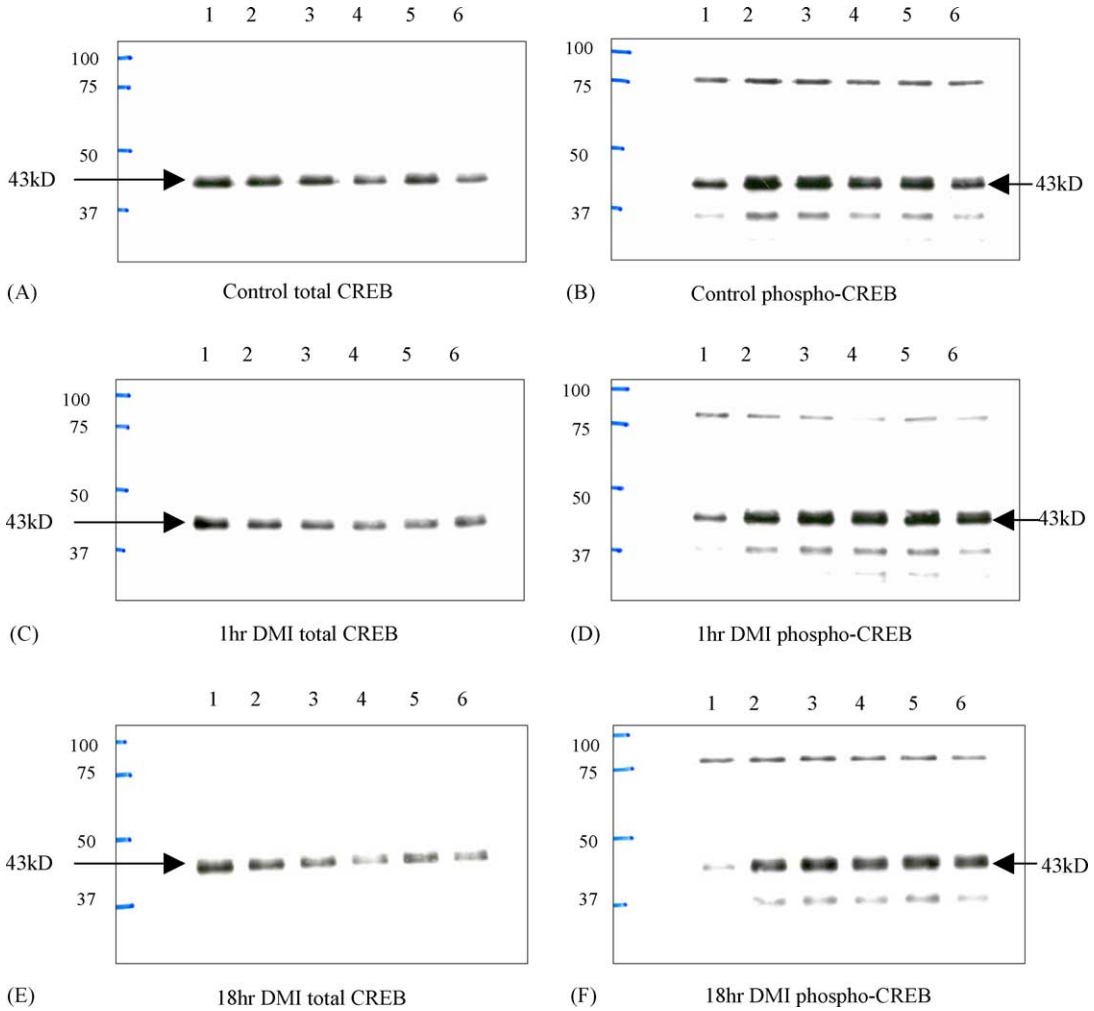

Fig. 7. Western blots for control and 20 μM DMI treated samples. Blots shown are from a single representative experiment ($n = 3$). On each blot the samples in each lane are: (1) basal, (2) 10 min isoprenaline, (3) 20 min isoprenaline, (4) 30 min isoprenaline, (5) 45 min isoprenaline, (6) 60 min isoprenaline. The bands indicated at 43 kDa are assumed to be either CREB (A, C, E) or phospho-CREB (B, D, F). According to the antibody data sheets supplied with the antibodies both the phosphorylated and unphosphorylated forms of CREB run as the same size. The upper band on the phospho-CREB blots are unidentified, however the lower band is likely to be phospho-ATF-1 or phospho-CREM, as they are also recognised by the phospho-CREB antibody and are part of the CREB family of proteins.

phosphatase reporter since parallel experiments, in which SPAP was measured in whole cell homogenates rather than in incubation medium, showed similar decreases (data not shown).

It is conceivable that the effect of DMI on isoprenaline-stimulated SPAP production could be due to antagonism of the β_2 receptor but this is unlikely since co-exposure of cells to DMI and isoprenaline without pre-incubation was without effect and the longer term effects of DMI were predominantly on the maximum response to the agonist rather than on the EC_{50}, ruling out competitive antagonism at least. In addition, DMI had an inhibitory effect on SPAP production stimulated by the adenylyl cyclase activator forskolin, suggesting a post-receptor target.

The inhibition of SPAP production seen after 18 h of DMI exposure was maintained for periods of up to 21 days without affecting cell viability. Generally, a greater interest has been shown in long-term adaptive responses to antidepressant agents in an attempt to relate these to the delayed clinical time course of the drugs. However, identification of an acute mechanism that initiates later adaptive responses is of equal importance. Noradrenaline and/or 5HT uptake inhibition are commonly considered to be crucial to antidepressant action [23,24] but CHO cells do not synthesise these monoamines rendering this mechanism irrelevant in relation to the effects reported here. Similarly, antidepressant-induced changes in glucocorticoid receptors, thought to be an important adaptive component [25] of their therapeutic action can be observed in vitro in primary blood cells lacking a neuronal-like expression of monoamines, their transporters and receptors, suggesting alternative acute mechanisms of antidepressant action [26].

The inhibitory effects of DMI on SPAP gene transcription do not appear to be mediated by altered cyclic AMP production or catabolism since there were no effects of the drug on isoprenaline or forskolin-stimulated [^{3}H]-cyclic AMP accumulation in [^{3}H]-adenine pre-labelled cells.

For SPAP gene transcription initiation, the cyclic AMP response elements (CREs) in the promoter region of the gene must be activated by phosphorylation of CRE binding protein (CREB). Phosphorylation of CREB at Ser_{133} is required for dimerisation and subsequent binding to CREs [27]. This can be achieved by protein kinase A (PKA) activated by increased intracellular cyclic AMP, but CREB activity can be further modulated by phosphorylation of additional sites on CREB or of other proteins associated with CREB [28]. For example increased intracellular Ca^{2+} can activate calcium/calmodulin kinases (CAMK) enabling CREB to be phosphorylated on its transcriptional regulatory site and allowing interaction with the CREB-binding protein (CBP/p300) [29]. Acute application, in vitro, of several classes of antidepressant drugs has been reported to inhibit Ca^{2+} related signalling [13,20,21,22,30] and Schwaninger et al. [13] concluded that antidepressant-mediated blockade of depolarisation-induced Ca^{2+} influx explained the inhibition of CRE-driven gene transcription in their study.

However, in the CHO cells used in the present study, isoprenaline activation of SPAP production was not accompanied by increased intracellular Ca^{2+} indicating that CREB phosphorylation is Ca^{2+}-independent in this model and that DMI could not be inhibiting SPAP gene transcription by limiting Ca^{2+} entry. Furthermore, Western blotting studies using an antibody specific for Ser_{133}-phosphorylated CREB showed no changes in CREB phosphorylation, nor was there a reduction in the expression of CREB in response to DMI treatment. This implies that the molecular target for DMI must lie downstream of CREB phosphorylation, possibly at the level of phospho-CREB binding to the CRE motifs in the DNA of the SPAP gene. However, Mayr et al. [31] reported that additional promoter-bound factors are required for target gene activation and DMI could interfere with the formation of such a complex. Whether this inhibitory action of DMI is common to naturally expressed CRE-driven genes remains to be determined.

The inhibition of SPAP gene transcription was not unique to DMI since similar effects were seen due to the selective serotonin reuptake inhibitor metabolite norfluoxetine; nor was it specific to drugs with antidepressant activity and the two neuroleptic agents chlorpromazine and clozapine also inhibited transcription. Previous reports have indicated that neuroleptics of different classes can acutely affect phosphorylation of CREB [32,33] in brain although the consequences for gene transcription in these more complex situations were not determined. It is possible that the antidepressants share a common site of action with the neuroleptic agents with regard to inhibition of cyclic AMP-driven gene transcription, but whether this is relevant to their respective therapeutic modes of action or side effects cannot be concluded from the data presented. The enhanced transcription seen in the presence of amphetamine is interesting but the mechanism was not investigated further.

There is clearly strong evidence for the involvement of the cyclic AMP signal transduction system in antidepressant drug action [5–11] and some of the most compelling of this indicates that, in vivo, antidepressants *up-regulate* CRE-driven gene transcription as well as the phosphorylation of CREB in several limbic brain regions [11]. Nevertheless, this could be the result of slowly developing, complex interactions of signalling pathways. It does not rule out the potential for one facet of the initial action of a variety of psychoactive drugs being an inhibition of the transcription of certain gene sets resulting in a reactive adaptive response characteristic of the individual drug and target cells with other properties of the drugs (e.g. interactions with receptors and transporters) contributing to their final pharmacological profile.

In summary, although it is accepted that the $CHO\beta_2$ SPAP cells are, at best, a pale reflection of neuronal cells in situ in the brain, the data presented demonstrate their utility as a model for investigations of psychoactive drug effects on cAMP-driven gene transcription. The findings also support the contention that adaptive changes can be provoked by a range of antidepressant drugs in the absence of changes in extracellular monoamines or Ca^{2+} channel activity and point to novel intracellular targets for these agents downstream of CREB phosphorylation.

Acknowledgements

The authors are grateful for the support of the Medical Research Council, the Hashemite University (Jordan) and the University of Nottingham.

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