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ORIGINAL INVESTIGATION

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Chromium treatment decreases the sensitivity of 5-HT_{2A} receptors

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Abstract *Rationale:* Recent case series suggest that chromium picolinate in doses of 400 µg daily may have antidepressant properties, perhaps through increasing the peripheral availability of tryptophan for brain serotonin (5-HT) synthesis. *Objectives:* To determine the effects of chromium treatment on plasma tryptophan availability and on brain 5-HT function in human and animal models. *Methods:* We studied the effects of short-term chromium supplementation on plasma concentrations of tryptophan and other large neutral amino acids. Brain 5-HT function was assessed by measuring the corticosterone/cortisol response to the 5-HT precursor, 5-hydroxytryptophan (5-HTP), a response believed to be mediated via indirect activation of 5-HT_{2A} receptors. *Results:* In rats, chromium increased peripheral and central tryptophan availability and elevated brain 5-HT content. Changes in peripheral tryptophan availability were not seen in humans but in both rats and humans, chromium lowered the cortisol response to challenge with 5-HTP. *Conclusions:* Chromium can modify brain 5-HT function in humans and animals, perhaps by altering the sensitivity of central 5-HT_{2A} receptors.

Keywords Chromium · Depression · 5-HT · Tryptophan · 5-$HT_{2A/2C}$ receptor · Cortisol

Introduction

Chromium is an essential trace element with an estimated daily intake in the Western diet of 25–50 µg (Anderson and Kovlovsky 1985). Chromium absorption is low, in the range of 0.4–2%, and the dietary intake of many people may be inadequate; however, estimation of nutritional chromium status is difficult because plasma levels of chromium correlate poorly with tissue stores (see Lukaski 1999). Chromium supplementation of the diet has been employed mainly to improve glucose tolerance in people with diabetes. The mechanism of this effect is not clear, but chromium may increase the activity of a low molecular weight chromium binding substance that enhances the effect of insulin by stimulating tyrosine kinase activity in insulin receptor protein (Davis et al. 1996). In general, chromium supplements in humans up to 1 mg daily appear safe and well tolerated, although there have been anecdotal accounts of renal impairment. Whether these are due to chromium is not established (Lukaski 1999).

Chromium has been postulated to have effects on both brain noradrenaline and serotonin (5-HT) function (see McCarty 1994). An effect of chromium on 5-HT function could be mediated via its ability to enhance the cellular effects of insulin. This would be expected to increase the entry of the 5-HT precursor tryptophan (TRP) into the brain by decreasing competition from other large neutral amino acids (NAAs) for transport at the blood-brain barrier. This in turn should lead to an increase in brain 5-HT synthesis (Fernstrom and Wurtman 1971).

More recently some case series have suggested that chromium in doses of approximately 400 µg daily may have potential as an antidepressant agent (McLeod et al. 1999; McLeod and Golden 2000). The aim of the present study was to characterise in both rats and humans the effects of chromium on TRP availability to the brain and on neuroendocrine measures of 5-HT neurotransmission.

Materials and methods

Animal study

Animals and chromium diet

Male Sprague-Dawley rats (Tuck, Battlebridge, UK) weighing 200–250 g were housed five to a cage in a light:dark cycle of 12:12 (0600–1800 hours). They had free access to food and water. The chromium (Cr^{3+}) diet and the equivalent control diet (RM-1) were obtained from Scientific Supplies Ltd (Witham, UK). The

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chromium diet consisted of RM-1 with 100 mg/kg chromium picolinate (Nutrition 22, Cardiff, UK).

Experimental methods

Animals received the chromium or placebo diets for 2 weeks prior to study. They were placed in the experimental room 2 h before testing. After this, animals were administered either 2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI, 2 mg/kg) or 5–hydroxytryptophan (5-HTP, 40 mg/kg) via the intraperitoneal (IP) route. The drugs were obtained from Sigma Poole, UK and were dissolved in saline and injected in a volume of approximately 0.25 ml (DOI) and 1.25 ml (5-HTP). Twenty minutes after injection animals were stunned and killed by decapitation. Trunk blood was collected into plain tubes and separated by centrifugation and stored at –40°C. For baseline biochemistry, animals were killed without receiving any drug administration.

Biochemical analysis

Plasma total corticosterone was measured by a single antibody coated-tube radioimmunoassay kit (DPC Ltd, Hengoed, UK). Brain 5-HT and 5-hydroxindoleacetic acid (5-HIAA) were measured using high performance liquid chromatography (HPLC) with amperometric end-point detection following extraction by homogenisation and deproteinisation using filtration (see Chi et al. 1999). Plasma and brain TRP were measured by HPLC with coulometric end-point detection. Samples were deproteinised with 3% trichloracetic and centrifuged, after which the supernatant was injected into the HPLC system. To obtain plasma free TRP, plasma was spin-filtered through a 12 K molecular filter. Plasma concentration of NAAs (leucine, isoleucine, valine, tyrosine and phenylalanine) were measured by an automated HPLC system with fluorescence end-point detection and pre-column sample derivatisation adapted from the method of Furst et al. (1990).

Data analysis

Differences between the experimental groups were assessed by the unpaired *t*-test (two tailed).

Volunteer study

Subjects and chromium treatment

We studied eight healthy subjects (seven male, one female) mean age 48 years (range 23–63 years) who had no current or past history of psychiatric disorder as assessed by the Structured Clinical Interview for DSM-IV (SCID). They had been free of psychotropic medication for at least 3 months. Subjects gave informed consent to the study, which was approved by the local ethics committee. Subjects took chromium picolinate (Boots, UK) 400 µg daily in the morning for 7–9 days.

Neuroendocrine testing

Each 5-HTP neuroendocrine test was placebo-controlled whereby subjects received 5-HTP and placebo in a single-blind, randomised, cross-over fashion, one day apart. Subjects were tested on two occasions (1) prior to chromium treatment (2) after 7–9 days of treatment. The female subject received her first and second 5-HTP tests in the early follicular phase of the menstrual cycle, with the start of chromium treatment being delayed so that this could be achieved. Subjects were tested after an overnight fast at approximately 0900 hours when an indwelling venous cannula was inserted and maintained with heparinised saline. Three baseline blood samples were taken over the next hour to measure cortisol and TRP availability, following which 5-HTP (100 mg orally) or placebo was administered. Subsequent blood samples were removed at 20-min intervals for the following 180 min for assay of plasma cortisol.

Biochemical analysis

Plasma was separated by centrifugation and stored at –30°C. Plasma cortisol was determined by radioimmunoassay (RIA). The intra- and inter-assay coefficients of variation over the range encompassed by the standard curve were 4.3% and 5.8%. Plasma TRP and NAAs were determined as described above.

Data analysis

Cortisol levels were analysed with a repeated measures analysis of variance (ANOVA) with "5-HTP" (5-HT versus placebo), "chromium" (before and during chromium treatment) and "time" (time before and after 5-HTP/placebo challenge) as the main within subject factors. The Huynh-Feldt correction was employed where the assumption of sphericity was violated. In addition, we subtracted the cortisol values during the placebo challenge from those during the 5-HTP challenge at each corresponding time point. These placebo-corrected cortisol responses to 5-HTP were analysed by a repeated measures ANOVA. The cortisol responses to 5-HTP and placebo were also measured as area under the curve (AUC) using the trapezoid method with subtraction of baseline secretion extrapolated from time "0". The placebo AUC was subtracted from the AUC following 5-HTP to give a placebo-corrected AUC value.

Results

Animal study

Compared to placebo, the 2-week chromium diet produced significant changes in plasma and brain TRP and brain 5-HT content (Table 1). Total plasma TRP was unchanged after chromium but free TRP was higher. In addition plasma concentrations of NAAs were lower resulting in an increased TRP:NAA ratio. Brain TRP and 5-HT concentrations were significantly increased by chromium. Chromium treatment significantly increased basal levels of corticosterone but diminished the corticosterone responses to 5-HTP and DOI (Fig. 1).

Table 1 Effect of a 2-week chromium diet (100 mg/kg) on measures of tryptophan (*TRP*) availability and 5-HT content. Results are mean±SEM from seven animals

	Control	Chromium
Total TRP (µg/ml)	18.8±1.0	17.6±0.8
Free TRP (µg/ml)	4.2±0.3	6.8±0.8**
NAAs (nm/ml)	1101±114	638±22***
Ratio TRP: NAAs	0.088±0.01	0.136±0.008**
Brain TRP (ng/g)	5.3±0.2	7.1±0.3***
Brain 5-HT (ng/g)	729±54	936±69*
Brain 5-HIAA (ng/g)	572±29	684±46

Significant differences from control diet, *$P<0.05$, **$P<0.01$, ***$P<0.001$

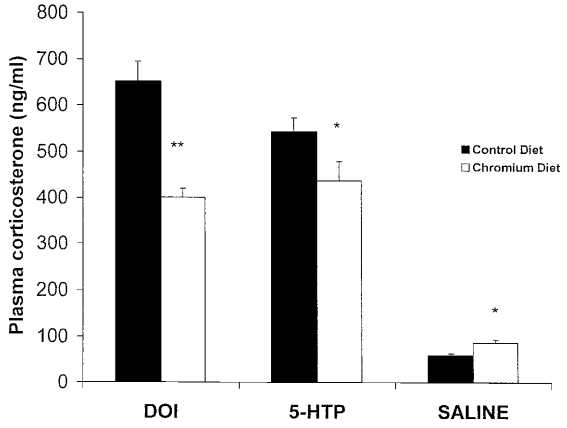

Fig. 1 Effects of DOI (2 mg/kg), 5-HTP (40 mg/kg) and saline injection on mean±SEM plasma corticosterone in groups of male rats (n=7) fed a chromium supplemented or control diet. Significantly different from control diet, *P<0.03; **P<0.02 (unpaired t-test)

Table 2 Effect of chromium treatment (400 µg daily for 7–9 days) on tryptophan (*TRP*) availability in volunteers[a]

	Pre-chromium	On chromium
Total TRP (µg/ml)	11.3±2.0	11.1±1.8
Free TRP (µg/ml)	0.71±0.23	0.77±0.26
NAAs (nm/ml)	519±93	540±66
Ratio TRP: NAAs	0.11±0.02	0.10±0.02

[a]Values are mean±SEM

Volunteer study

In contrast to the findings in animals, a one week treatment period with chromium did not alter free or total plasma TRP or plasma NAAs (Table 2). However, the cortisol response to 5-HTP was lower after chromium treatment (Fig. 2).

5-HTP increased plasma cortisol as shown by a significant interaction between 5-HTP and time on the ANOVA (F=6.23; df=4,25; P=0.002). By itself chromium did not alter cortisol. Thus the ANOVA showed no significant main effect of chromium (F=0.24; df=1,7; P=0.64) or any interaction between chromium and time (F=1.95; df=8,54; P=0.07). There was, however, a significant three-way interaction between 5-HTP, chromium and time (F=2.68; df=6,42; P=0.027). The placebo-corrected 5-HTP values also showed a significant interaction between chromium and time (F=2.61; df=6,42; P=0.03). Analysis of the placebo challenge data alone showed no main effect of chromium (F=0.01; df=1,7, P=0.98) on and no interaction between chromium and time (F=1.98, df=4,30, P=0.12). Finally, the placebo corrected AUC values for 5-HTP-induced cortisol release were significantly lower after chromium treatment (mean±SEM pre-chromium=1030±283 versus –262±271 mcg×min/100 ml, P=0.048). These data indicate that chromium treatment lowered the cortisol response to 5-HTP.

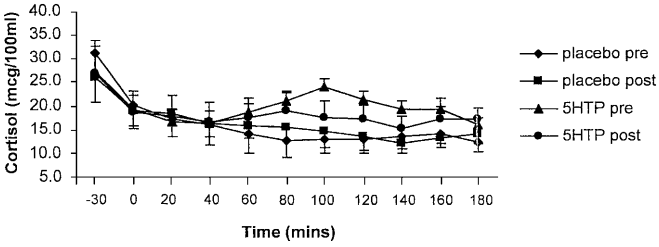

Fig. 2 Mean±SEM plasma cortisol levels before and following 5-HTP (100 mg given orally at time "0") or placebo in eight volunteers. Subjects were studied with 5-HTP/placebo challenges on two occasions, before chromium treatment and 7–9 days following chromium. Placebo corrected AUC values for 5-HTP-induced cortisol release were significantly lower after chromium treatment, P=0.048, paired t-test (for ANOVA results see text)

Discussion

Our findings indicate that chromium treatment modifies aspects of brain 5-HT function in both humans and animals. The animal studies suggest that chromium may alter 5-HT neuroendocrine function via effects on peripheral TRP availability. In human volunteers, however, a similar decrease in 5-HTP-mediated cortisol release was apparent in the absence of changes in TRP availability.

The common finding in both animals and humans was a decreased corticosterone/cortisol response to 5-HTP. Direct stimulation of several post-synaptic serotonin receptors (5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}) facilitates corticosterone release (Cowen 1993), but studies with selective 5-HT receptor antagonists suggest that in both rat and human 5-HTP-induced increases in cortisol/corticosterone are mediated via indirect activation of 5-HT_{2A} receptors (Gartside and Cowen 1990; Lee et al. 1991; Meltzer and Maes 1994). Similarly, although DOI binds to both 5-HT_{2A} and 5-HT_{2C} receptors, the corticotropin responses to DOI in the rat appear to be mediated exclusively via 5-HT_{2A} receptors (Gartside et al. 1992; Van de Kar et al. 2001). Overall, therefore, our findings suggest that chromium impairs 5-HT_{2A} receptor-mediated corticosterone/cortisol release, but a role for 5-HT_{2C} receptors cannot be completely excluded by the present data. Further studies with more selective 5-HT_{2A} and 5-HT_{2C} receptor ligands would help clarify this issue.

The effects of antidepressant treatment on 5-HTP-mediated cortisol release have not been studied extensively in humans, although we have found previously that short-term (7 days) treatment with SSRIs in healthy volunteers produces a substantial increase in 5-HTP-mediated cortisol release (Sargent et al. 1998). There is evidence, however, that some antidepressants such as imipramine, which possess 5-HT_2 receptor antagonist properties, lower the cortisol response to 5-HTP (Meltzer et al. 1984).

In rats, chromium appeared to produce striking increases in TRP availability. Both plasma free TRP and the ratio of plasma total TRP to competing NAAs have been proposed as important determinants of brain TRP

entry (see Curzon 1979). Chromium has been suggested to facilitate the cellular effects of insulin (see McCarty 1994), and this would be consistent with lowered plasma concentrations of NAAs. In addition, however, chromium increased plasma free TRP. We have no explanation for this finding, which was unexpected. However, consistent with the predicted increase in peripheral TRP availability, chromium increased TRP and 5-HT content in the rat brain.

The changes in TRP availability in rats and the presumably resultant increases in brain 5-HT content could have produced an adaptive down-regulation of post-synaptic 5-HT_2 receptors. This might account for the decrease in cortisol response to the 5-HT_2 receptor agonist, DOI. In humans, however, chromium did not alter TRP availability but still lowered the cortisol response to 5-HTP. We believe that the most likely explanation is that chromium treatment lowers the sensitivity of post-synaptic 5-HT_{2A} receptors independent of its actions on peripheral TRP availability, although the mechanism of this effect remains to be determined. It is, however, of interest that 5-HT_{2A} receptors have been shown to modify the cellular uptake of glucose (Hajduch et al. 1999) and chromium may improve glycaemic control in patients with diabetes (Mertz 1993).

With neuroendocrine measures of brain 5-HT function, it is possible that changes in endocrine response may be caused by alterations in the regulation of the hormone concerned rather than that of 5-HT (see Cowen 1998). In the rat, for example, it is possible that elevated baseline levels of corticosterone could have lowered the corticosterone response to 5-HTP and DOI by increasing feedback at pituitary and hypothalamus. In humans, however, chromium caused a similar blunting in 5-HTP-induced cortisol release without elevating baseline cortisol levels. Nevertheless, it is possible that chromium might alter HPA axis function in humans without producing obvious effects on short-term sampling of plasma cortisol.

The differing effects of chromium on TRP availability in rat and human require explanation. The dose of chromium given to the rats was substantially more than that given to the volunteers. Another factor that might be relevant is that the treatment period in humans was shorter. An ability of chromium to increase TRP availability in humans would be an important pointer to possible therapeutic uses. Further work will be required to see if such an effect can be demonstrated at clinically acceptable doses.

In conclusion, our findings show that short-term administration of chromium to both humans and animals appears to decrease endocrine responses to 5-HT_{2A} receptor stimulation. The mechanism of this effect requires further study and may, for example, involve change in receptor expression, signal transduction or intracellular messengers. Interestingly, reductions in 5-HT_{2A} receptor sensitivity are also seen following chronic treatment with certain antidepressant drugs (Meltzer and Maes 1984; Gartside et al. 1992; Maj et al. 1996) and is therefore conceivable that 5-HT_{2A} receptor downregulation could be related to the antidepressant effects of chromium in depressed patients. The effects of chromium on mood may, of course, be particularly relevant to patients with diabetes where depressive symptomatology has been shown to correlate with both glycemic control and the extent of diabetic complications (De Groot et al. 2001; Van Tilburg et al. 2001).

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