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Enhancement of serotonin uptake by cortisol: A possible link between stress and depression

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Stress and depression are characterized by elevation of circulating cortisol, as well as by changes in physiological functions. In this study, we addressed the possibility that elevated cortisol is also associated with the origin and development of depression. We report here that cortisol at the nM–μM concentration range induces a substantial increase in serotonin uptake both in vitro, by human peripheral blood lymphocytes (PBLs) and cortical neuronal cells, and in vivo, by rabbit PBLs, owing to promotion of synthesis of the serotonin transporter. These findings offer a novel molecular mechanism for depression associated with stress. Accordingly, the elevated cortisol induced by stress increases serotonin uptake, under both rest and nerve stimulation, which is overtly expressed in symptoms of depression.

Mental stress activates a series of physiological systems as part of the adaptive response. These include the immediate release of catecholamines, noradrenaline, and adrenaline in the central nervous system (CNS) and the autonomic nervous system (ANS), as well as the activation of the limbic hypothalamo-pituitary-adrenal (HPA) system (Akil & Morano, 1995; Chrousos & Gold, 1992). The HPA axis, also known as the stress axis (Selye, 1946, 1950), takes part in the secretion of the corticotropin-releasing hormone (CRH) and the adrenocorticotropin hormone (ACTH; Antoni, 1986), which activate the biosynthesis of glucocorticoids, in particular cortisol, by cells of the adrenal cortex (Axelrod & Reisine, 1984). The action of cortisol then proceeds by diffusion through the plasma membrane of the target cell, followed by binding to the intracellular mineralocorticoid receptors (MRs) and the glucocorticoid receptors (GRs; Evans, 1988; Truss & Beato, 1993). At low levels, cortisol predominantly binds to the MRs, whereas at higher levels, such as those prevailing in stress, cortisol will progressively bind to the GRs (Oitzl, van Haarst, & de Kloet, 1997). Upon cortisol binding, the GR undergoes a conformational change, which facilitates binding to DNA (Beato, 1989; Beato, Chalepakis, Schauer, & Slater, 1989). The GR–cortisol complex thus regulates transcriptional responses by binding to a hormone response element (HRE) on the promoter region of the corresponding genes (Reichel & Jacob, 1993; Scheidereit et al., 1986).

Homeostasis of glucocorticoids within the physiological range, which counteracts the acute response to stress, operates predominantly through the HPA axis (Munck, Guyre, & Holbrook, 1984). This control mechanism involves multiple negative feedback loops, mediated mainly by the steroids themselves (Dallman et al., 1994; Dallman, Makara, Roberts, Levin, & Blum, 1985; Keller-Wood & Dallman, 1984). However, under chronic stress, the HPA system is dysregulated, resulting in pathophysiological changes that may develop into various types of disorders (Selye, 1946, 1950)—in particular, depression. A significant association between stress and depression is now well documented (Abramson, Seligman, & Teasdale, 1978; Gold, Goodwin, & Chrousos, 1988a; Holsboer, 1995; Post, 1992), and for both syndromes, it is characterized by hypercortisolism (Gold, Goodwin, & Chrousos, 1988b; Mokrani, Duval, Crocq, Bailey, & Macher, 1997; Murphy, 1991; Peeters & Broekkamp, 1994).

Ample evidence indicates that dysfunction of the serotonergic system in the CNS underlies the origin and development of depression (Henninger, 1995; Maes & Meltzer, 1995; Meltzer & Lowy, 1987; Owens & Nemeroff, 1994). At the neuronal level, termination of the serotonergic neurotransmission is achieved by the rapid clearance of the neurotransmitter from the synaptic cleft, partly through oxidation by monoamine oxidase A and partly by reuptake by the serotonin transporter (Amara & Kuhar, 1993; Barker & Blakely, 1995). These activities determine the effective concentration of serotonin at the synaptic cleft and its availability for the activation of both pre- and postsynaptic receptors (Amara & Kuhar, 1993; Barker & Blakely, 1995). The serotonin transporter is therefore of great interest in psychiatry, and as such it is the target of most antidepressants, including tricyclics and selective-serotonin-reuptake-inhibitors (Hoffman, Mezey, & Brownstein, 1991; Kanner & Schuldiner, 1987).

The gene encoding for the human serotonin transporter was shown to be identical in neurons and platelets

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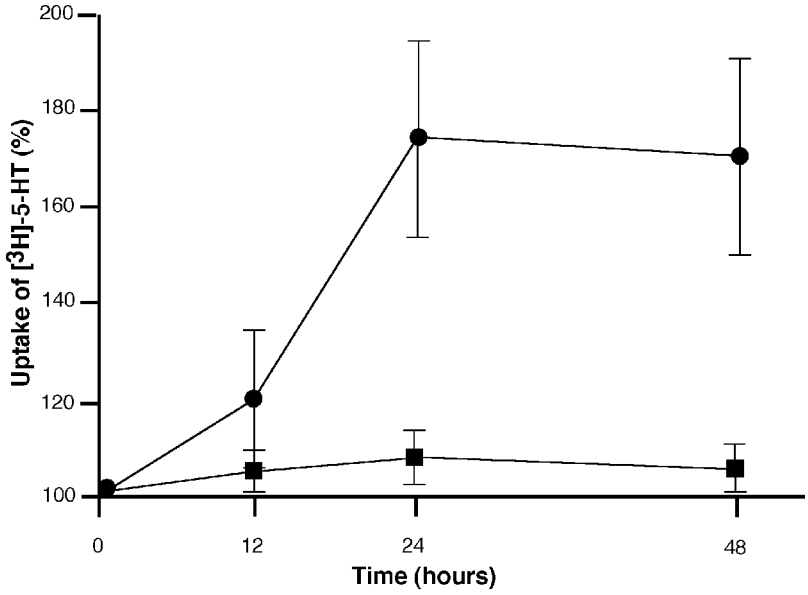


Figure 1. Effect of cortisol on the uptake of serotonin by human peripheral blood lymphocytes (PBLs) (●) and platelets (■) obtained from freshly drawn blood of 6 healthy donors. 10^7 PBLs or 10^8 platelets were incubated in triplicates of 1-ml medium for up to 48 h in the absence or presence of 10^{-8} M cortisol. The cells were pulsed at different times with 10^{-6} M tritiated serotonin ([^{3}H]-5-HT) and incubated for 15 min at 37ºC, then harvested and scored for radioactivity incorporation. The results are presented as the mean ± *SD* of percentage of increase of uptake in the samples containing cortisol. The increase in [^{3}H]-5-HT uptake after 24 and 48 h of treatment with cortisol was highly significant ($p < .001$; statistical details are given in the text).

(Lesch et al., 1994; Lesch, Wolozin, Murphy, & Reiderer, 1993; Ramamoorthy et al., 1993). More recently, the serotonin transporter of human peripheral blood lymphocytes (PBLs) was identified and was also found to be identical to that of the neuronal tissues (Faraj, Olkowski, & Jackson, 1994, 1997). In the following study, we demonstrate that the presence of cortisol in the interstitial fluid, at levels that prevail in stress, induces a substantial increase in serotonin uptake, owing to induction of synthesis of the serotonin transporter. This observation may have a direct implication for depression that follows stress conditions.

METHOD

Blood samples of 50 ml obtained from 6 healthy normal volunteers were supplied from a local blood bank immediately after drawing. The samples were then centrifuged at 900 rpm for 20 min, to separate the platelet rich plasma (PRP). The number of platelets in the PRP was counted with a hemocytometer and adjusted with phosphate buffered saline (PBS) to 1×10^8 platelets/ml. The remaining blood fraction was layered over 10 ml of Ficoll-Paque (Pharmacia) and centrifuged at 1,800 rpm for 21 min. The mononuclear leucocyte layer, containing approximately 80% lymphocytes, was further processed for lymphocyte purification, as has been described (Faraj et al., 1994, 1997). The final lymphocyte sample was resuspended in complete medium (composed of RPMI-1640 medium containing 10% FCS, 2 mM L-glutamine, 1 mM sodium pyruvate, nonessential amino acids, and antibiotics), and the number of lymphocytes was adjusted to 1×10^7 cells/ml. Viability of the isolated lymphocytes, assessed by trypan blue exclusion, was higher than 90%. The lymphocytes were distributed in six well plates (Falcon), 1 ml per sample, and maintained in a 5% CO_2 humidified incubator at 37°C.

Uptake of serotonin (5-HT) was assayed with tritiated 5-HT ([^{3}H]-5-HT) essentially as has been reported (Faraj, Olkowski, & Jackson, 1991, 1994). In assays of the effect of cortisol on the uptake of 5-HT, cortisol-treated and control samples were incubated in triplicate for 15 min at 37°C in the presence of 10^{-6} M 5-HT (Sigma), doped with [^{3}H]-5-HT (Amersham), in six Falcon well plates. The uptake was terminated by centrifugation at 14,000 rpm for 5 min, at 4°C, in an eppendorf microfuge. The pellet was washed with 1 ml of ice-cold PBS, centrifuged, suspended in 300 μl of 1M NaOH, incubated overnight at 37°C, and neutralized with 30 μl of concentrated HCl. The homogenates were transferred to scintillation vials, containing 3 ml of scintillation fluid, and scored for radioactivity. The dose dependency of the cortisol effect on the uptake of 5-HT by human lymphocytes was tested with decreasing concentrations of cortisol (10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, and 10^{-10} M) after incubation for 24 or 48 h. Nonspecific accumulation of [^{3}H]-5-HT was determined by preincubation of samples in the presence of 10^{-4} M clomipramine, a potent inhibitor of specific 5-HT uptake (Thase & Rush, 1995).

The effect of cortisol on the uptake of 5-HT by human platelets was tested with a constant concentration of cortisol (10^{-8} M) for increasing periods of time (1, 12, 24, and 48 h). Cortisol-treated and control platelet samples were incubated in duplicates for 15 min at 37°C in the presence of 10^{-6} M of [^{3}H]-5-HT and were processed as described above.

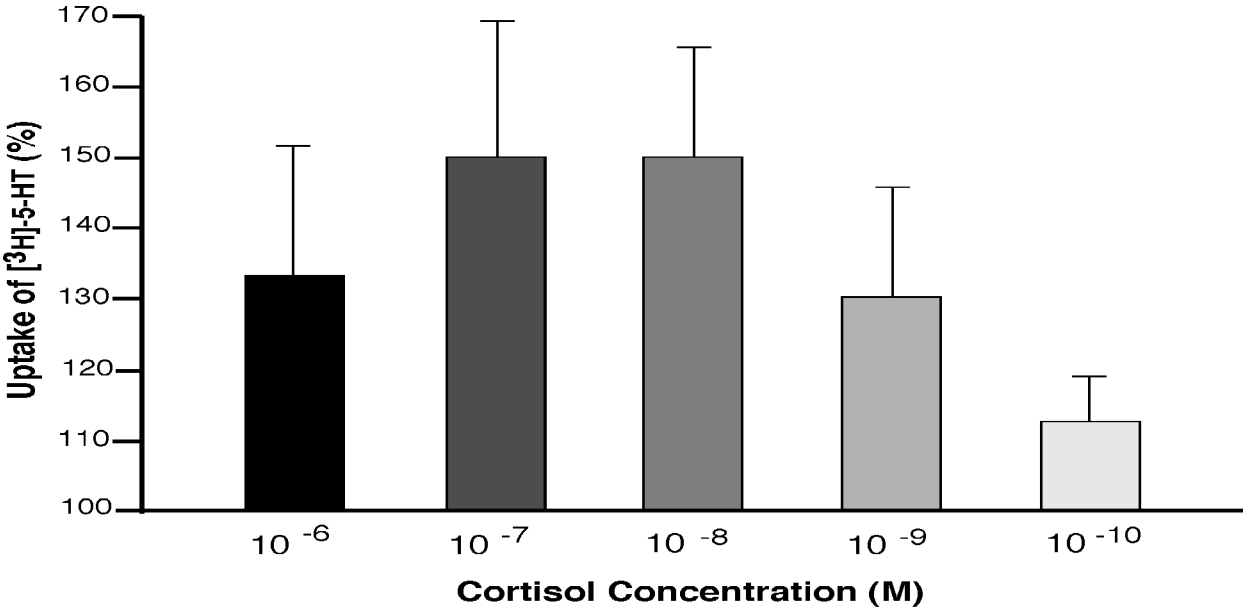


Figure 2. Stimulation of [^{3}H]-5-HT uptake by human peripheral blood lymphocytes after 24 h incubation with increasing amounts of cortisol. Conditions are as described in the legend to Figure 1.

The neuronal cell lines 5H-SY5Y, CG, TE-6F1, and HCN-1 were passaged in vitro under standard tissue culture conditions.

For bolus injection in rabbits (New Zealand White; age, 6 months), 2×10^{-4} M of cortisol in ethanol was diluted 1:100 into sterile saline with vortexing. Two milliliters of the 2 μM cortisol was injected intravenously (i.v.) immediately after preparation.

The comparative mRNA contents of serotonin transporter and β-actin (control) were measured by reverse transcription–polymerase chain reaction (RT–PCR). Total RNA from cortisol-treated and untreated lymphocytes was extracted by Tri-Reagent (MRCI) and was reverse transcribed (RT) to cDNA. PCR amplification of the human serotonin transporter cDNA was performed using 1 μl total cDNA. The primers were a sense (5′- G A C A C A C G G C A C T C T A T C C C -3′) and an antisense (5′- G G T G C A G T T G C C A G T G T T C C -3′). PCR amplification of the human β-actin cDNA, utilized as an internal control, was performed analogously, using the respective sense (5′- C T A T C C C T G T A C G C C T C T G G -3′) and an antisense (5′- G A G G G G A A A T G A G G G C A G G A C -3′) primers. Products were separated on 1.2% agarose gels, stained with ethidium bromide, and visualized under UV. The staining intensity was scored by Molecular Analyst software (BioRad).

The significance of the difference between groups was analyzed by a repeated measures analysis of variance (ANOVA), using the computer program Statistica 5.0. The results are expressed as the mean ± the standard deviation (*SD*) of the percentage of basal values, in addition to *F* values, degrees of freedom (*df*), and *p* values.

RESULTS

The first set of experiments was conducted with PBLs of 6 healthy donors, in which the in vitro effects of cortisol on the uptake of serotonin by PBLs were determined. Incubation of PBLs in the presence of 10^{-8} M cortisol, for increasing periods of time, produced a highly significant stimulatory effect on the uptake of serotonin. Figure 1 presents the effect of cortisol treatment on serotonin uptake at different incubation times in PBLs and platelets. An ANOVA statistical analysis indicated a highly significant difference between groups ($df = 1$, $F = 219.4$, and $p < .001$, for group main effect) and times ($df = 3$, $F = 52.8$, and $p < .001$) and a group × time interaction ($df = 3$, $F = 36.3$, and $p < .001$). The effect of cortisol on the uptake of histamine was less pronounced, and almost no effect on the uptake of dopamine was observed (data not shown). Upon incubation of PBLs for 24 h with different concentrations of cortisol, a maximal stimulatory effect of serotonin uptake was observed in the presence of 10^{-7}–10^{-9} M cortisol (Figure 2). The effect of cortisol on the uptake of serotonin by human platelets was examined analogously. As is shown in Figure 1, no significant change in the uptake of serotonin by human platelets upon preincubation with 10^{-8} M cortisol was observed.

The effect of cortisol on the amount of the serotonin transporter mRNA was analyzed by quantitative RT–PCR. Upon incubation of PBLs for 48 h in the presence of cortisol, a significant increase in the serotonin transporter mRNA was observed (Figure 3). In comparison, no increase was observed in the amount of β-actin mRNA. Sequence analysis of the amplified band indicated complete homology to the nucleotide sequence of the serotonin transporter. It should be noted that the increase in mRNA of the serotonin transporter presented in Figure 3 amounts to 2.5-fold, whereas the increase in the overt serotonin uptake in the same PBLs sample was 1.5-fold.

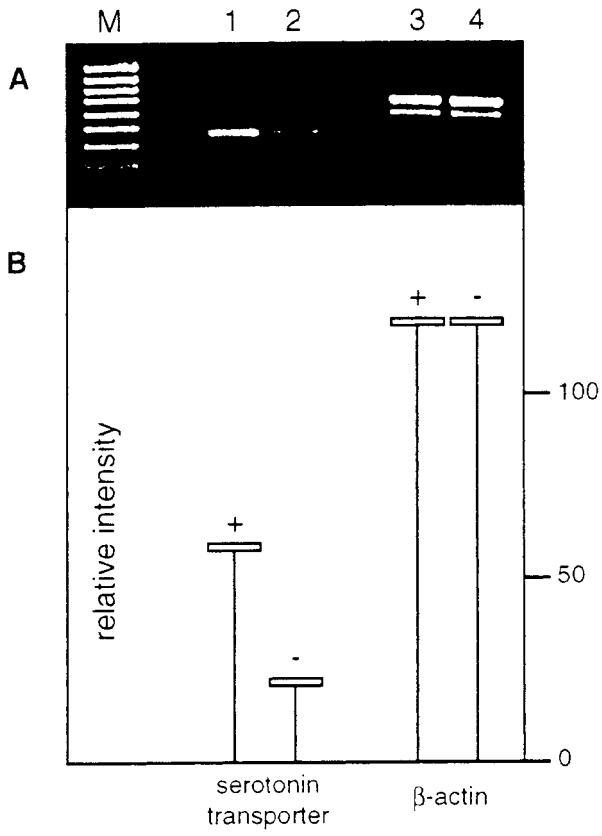


Figure 3. (A) Polymerase chain reaction (PCR) analysis of the human serotonin transporter and β-actin mRNAs. Total RNA was extracted from human lymphocytes, reverse transcribed, and PCR amplified. Lane M: 100 bp-ladder DNA marker (MBI); Lanes 1 and 3: PCR products of serotonin transporter and β-actin, respectively, from cells incubated for 48 h in the presence of 10^{-8} M cortisol; Lanes 2 and 4: PCR products of serotonin transporter and β-actin, respectively, from untreated cells. (B) Relative intensity of the PCR products evaluated by densitometry (Analyzer, Bio-Rad) of the bands corresponding to the serotonin transporter (Lanes 1 and 2), and to its respective β-actin control (Lanes 3 and 4).

This difference could be accounted for by incomplete translation or by a fraction of the induced serotonin transporter that did not integrate into the plasma membrane. The ensuing increase in the membrane expression of the serotonin transporter could, therefore, account for the observed increase in serotonin uptake induced by cortisol.

To select an adequate model for neuronal cells, we first tested 5-HT uptake in the human neuroblastoma SH-SY5Y, the rat glioma C6, the human medulloblastoma TE-671, and the human cortical neuronal cells HCN-1 (Dunn, Perez-Polo, & Wood, 1996; Ronnett, Hester, Nye, Connors, & Snyder, 1990). Only the HCN-1 cells exhibited an appreciable level of 5-HT uptake activity, similar in magnitude to that observed in human PBLs. The elevation of 5-HT uptake in HCN-1 cells by decreased concentrations of cortisol is presented in Figure 4. The induction of serotonin transporter synthesis in the HCN-1 upon 48-h incubation with 10^{-7} M cortisol was assayed by RT–PCR, as with PBLs (Figure 3). The results reflected an increase in the mRNA of the serotonin transporter by approximately 20%–40% (data not shown).

Systemic in vivo effect of cortisol was tested in the rabbit, since in this particular animal, the function of cortisol is similar in physiological patterns to those in man (Havenaar, Meijer, Morton, Ritskes-Hoitinga, & Zwart, 1993). As is shown in Figure 5, before injection of cortisol, the PBLs of each individual rabbit responded in vitro to the presence of cortisol by an increase in serotonin uptake of 20%–80%, a range similar to that observed with human PBLs (Figures 1 and 2). Moreover, the increase in serotonin uptake in the two consecutive measurements before cortisol injection were close, which indicated that the blood drawing per se made no practical contribution to the observed induction of 5-HT uptake. The bolus injection of cortisol reduced markedly the subsequent in vitro effect of cortisol after 24 h. This observation clearly indicates that the PBLs drawn at that time were already furnished with excess serotonin transporters. This excess vanished 6 days after the cortisol injection (Figure 5), suggesting that time homeostasis had reached an overall recuperation of the short-term effect of cortisol.

Cortisol was shown to mediate a decrease in membrane fluidity of endothelial cells (Gerritsen, Schwarz, & Medow, 1991). We have therefore determined the change in membrane fluidity of lymphocytes after 48-h incubation with 10^{-8} M cortisol, as in the 5-HT uptake experiments, by the well-known method of fluorescence depolarization with DPH as a membrane probe (Shinitzky & Barenholz, 1978). A clear decrease in membrane fluidity by 23%–35% was observed, which is similar in magnitude to the reported cortisol-induced decrease in membrane fluidity of endothelial cells (Gerritsen et al., 1991). However, a decrease in membrane fluidity is, in general, accompanied by a decrease in carrier-mediated transport (Shinitzky, 1984), including that of 5-HT (Block & Edwards, 1987). We could thus exclude the possibility that changes in membrane fluidity could account for the observed increase in 5-HT uptake.

The effect of cortisol on the mitogenic response of lymphocytes was assayed independently. Lymphocytes were incubated with different concentrations of cortisol (10^{-8}, 10^{-9}, and 10^{-10} M), with or without 5-HT. Stimulation of the lymphocyte samples was carried out with the mitogen phytohemagglutinin (PHA) for 5 days at 37°C. For the last 18 h, each well was pulsed with 1 μCi [^{3}H]-thymidine, followed by harvesting and scoring of radioactivity. PHA induced a marked stimulation (about 20-fold) of [^{3}H]-thymidine uptake, whereas in the presence of cortisol, even at a concentration of 10^{-10} M, this effect was reduced by up to 90%. However, the enhancement of 5-HT uptake by PBLs was observed in the PHA-stimulated and PHA + cortisol samples.

DISCUSSION

Major depression is a syndromal disorder, characterized by psychological symptoms and biological alter-

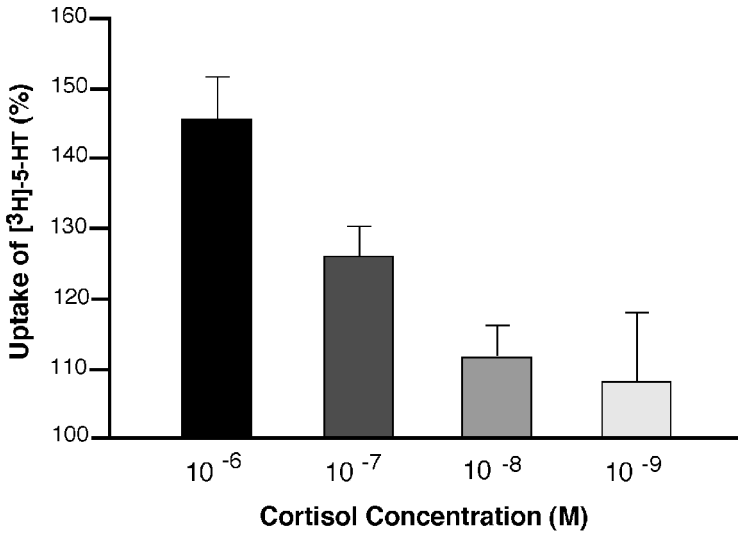
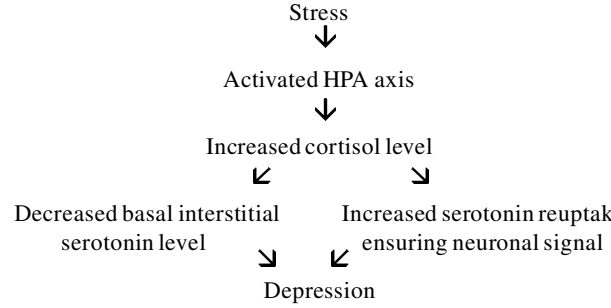


Figure 4. Stimulation of [^{3}H]-5-HT uptake by the human cortical neuronal cell line HCN-1. The cells were incubated in triplicates of 10^7 cells per milliliter for 48 h in the absence or presence of decreasing concentrations of cortisol, then pulsed with 10^{-6} M [^{3}H]-5-HT and incubated for 15 min at 37°C. The results are presented as the mean ± *SD* of the percentage of increase of [^{3}H]-5-HT uptake in the samples containing cortisol.

ations. The role of serotonin in the pathophysiology of major depression has been extensively studied, giving rise to and further supporting the serotonergic hypothesis of major depression (Chaouloff, 1993; Henninger, 1995; Maes & Meltzer, 1995; Meltzer & Lowy, 1987; Owens & Nemeroff, 1994), which postulates that a deficient serotonergic activity in the CNS implicates a higher vulnerability to this disorder.

The results presented in this study indicate that cortisol at concentrations of around 10 nM can induce a significant increase in serotonin uptake by lymphocytes (Figures 1 and 2) and neuronal cells (Figure 4). This increase is associated with the induction of synthesis of the serotonin transporter (Figure 3), which may then integrate into the cell plasma membrane and thus elevate the level of serotonin transport. Two further observations support our finding that cortisol induces an increase in the level of operating serotonin transporters. The overt effect of cortisol on the 5-HT uptake was not instantaneous but emerged only after a few hours of incubation and reached a maximum after approximately 24 h (Figure 2). In addition, cortisol had no effect on the uptake of 5-HT by platelets, which lack the transcriptional apparatus for protein synthesis.

The most intriguing question that arises from the above observations is whether the results with lymphocytes or neuronal cells reflect the situation in the synaptic cleft. In other words, does cortisol enhance the reuptake of 5-HT during the production of the neuronal signal. A series of comparative studies indicate that the analogy between neurotransmitter uptake by lymphocytes and neurons is actually valid (Faraj et al., 1991, 1994, 1997; Grodzicki et al., 1990; Halbach & Henning, 1989; Le Fur, Phan, & Uzan, 1980; Mann et al., 1985). Such a putative enhancement of 5-HT uptake in the synaptic cleft, induced by elevated extracellular cortisol, could provide a hypothetical molecular setting for a mechanism describing how stress can induce depression (Abramson et al., 1978; Gold et al., 1988a; Holsboer, 1995; Post, 1992; Teasdale, 1978). This mechanism is presented schematically in the following diagram and is further delineated in Figure 6. Accordingly, stress-induced elevated cortisol reduces the tonic level of serotonin in the synaptic cleft and stimulates its reuptake after neuronal impulse. These two processes presumably contribute to the overt symptoms of depression at rest and upon external stimulus.



Several lines of indirect evidence converge to support the proposed notion of a direct correlation between increased cortisol level and impaired serotonergic function in depression. In general, patients with major depression have a high level of plasma cortisol (Carroll, Curtis, &

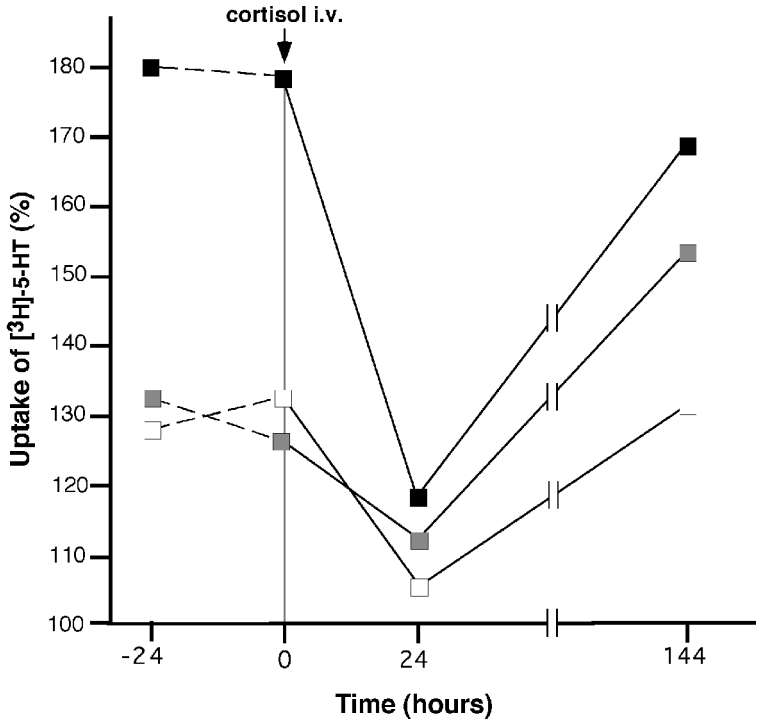


Figure 5. Increase in serotonin uptake by peripheral blood lymphocytes (PBLs) of 3 individual female NZW rabbits after 24 h incubation in vitro with 10^{-8} M cortisol, before (- - -) and after (—) bolus intravenous (i.v.) injection of 2 ml 2 μM cortisol in PBS. For each [^{3}H]-5-HT uptake test, 8 ml of blood was drawn from the right ear and PBLs were isolated and divided to triplicate settings. Each PBL sample was then tested for the in vitro effect of 10^{-8} M cortisol on [^{3}H]-5-HT uptake. Bolus i.v. injection of cortisol was carried out in the left ear, 1 h after the second blood drawing. The results are presented as the triplicate mean values, for which *SD* in all cases was less than $\pm 6\%$.

Mendels, 1976; Charles et al., 1986; Gold et al., 1988b; Mokrani et al., 1997; Murphy, 1991). Normalization of their blood cortisol levels, usually correlates with successful clinical treatment and good prognosis (Amsterdam, Winokur, Caroff, & Conn, 1982). Furthermore, hypercortisolemic depressed patients treated with antiglucocorticoid interventions, in general, experience alleviation of their depressive symptomatology (Murphy, Dhar, Ghadirian, Chouinard, & Keller, 1991; Reus, Wolkowitz, & Frederick, 1997; Wolkowitz et al., 1993). More support for the above scheme is reflected in patients of Cushing's syndrome, who carry a high level of cortisol and, in general, present symptoms of depression (Kelly, Checkley, & Bender, 1980; Starkman, Schteingart, & Schork, 1981). It was recently suggested that the mechanism of action of antidepressants may involve a stimulation of corticosteroid receptor expression, resulting in a negative feedback response to cortisol, expressed in alleviation of affective symptoms (Barden, Reul, & Holsboer, 1995).

The principal components of the adaptive response to stress have been shown to operate in consecutive stages, through a consistent pattern of activation of the catecholaminergic systems and the HPA axis. Upon exposure to stress, catecholamines are released immediately to reach postsynaptic target tissues and trigger second-messenger cascades within seconds. Cortisol is then released within minutes, and its final effect is expressed after hours, since it involves transcriptional events. This difference in time course is relevant for understanding the mechanisms underlying the different consequences of acute and chronic exposure to stressors (McEwen & Sapolsky, 1995).

The syndrome of major depression, and particularly the melancholic type, seems also to represent dysregulation of the adaptive response to stress (Chrousos & Gold, 1992)—particularly, disinhibition of the HPA axis (Akil & Morano, 1995; Peeters & Broekkamp, 1994). The consequent hypercortisolemia, observed in most patients suffering from major depression (Carroll et al., 1976; Charles et al., 1986), represents one of the most consistent findings in biological psychiatry (Gold et al., 1988a, 1988b; Mokrani et al., 1997; Murphy, 1991, 1997). The HPA axis is known to be regulated by limbic structures in the central nucleus of the amygdala. These structures are involved in the activation of the characteristic neuroendocrine responses to stress (LeDoux, Iwata, Cicchetti, & Reis, 1988) and inhibitory input from the hippocampus (Herman et al., 1989; McEwen & Brinton, 1987; Smelik, 1987), which has been shown to be enervated by seroton-

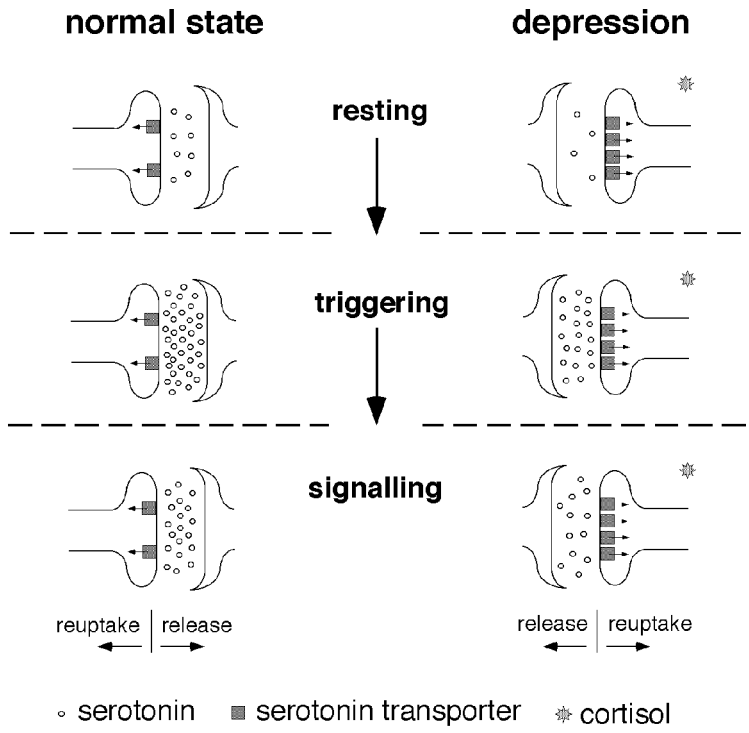


Figure 6. Hypothetical description of how elevated cortisol in depression can induce reduction of serotonin in the synaptic cleft, both at rest and upon neuronal activation.

ergic input from the median raphe-forebrain tract (Deakin, 1998). It has been proposed that hypercortisolemia mediates the mood changes observed in depression (Peeters & Broekkamp, 1994) through these reciprocal interactions between the HPA axis and the serotonergic system.

Our hypothesis that cortisol, by enhancing the expression of the serotonin transporter, could down-regulate the availability of serotonin in the synaptic cleft, leading to defective serotonergic neurotransmission in the CNS, may provide a biological link for better understanding the origin of depression induced by chronic stress. This proposed mechanism, where depression is attributed to hypercortisolemia, produced by dysregulation of the HPA axis during chronic stress situations, is schematically delineated in Figure 6. It implies that one of the targets of antidepression treatments could be in the direction of normalization of the HPA system. This could be achieved either directly by psychopharmacological therapies or indirectly by psychotherapeutic approaches, which may reduce cortisol by reinforcing the controllability of stressful situations.

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