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Dexamethasone Reduced Clonidine-induced Hypoactivity in Mice

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Abstract

Reduced clonidine anti-nociception in mice given low doses of dexamethasone has encouraged us to investigate the effects of dexamethasone pretreatment on locomotor hypoactivity, another example of clonidine-induced behaviour in mice.

Dexamethasone administered intraperitoneally (0·1, 1·0, 10 mg kg^{-1}) 30 min before clonidine reduced clonidine-induced locomotor hypoactivity in the activity cage to an extent which was dose-dependent. Dexamethasone administered centrally (10 ng/mouse) 30 min before clonidine was also able to reduce clonidine-induced locomotor hypoactivity. Cycloheximide administered at a dose of 10 mg kg^{-1} 2 h before clonidine did not change the effects of clonidine but was able to prevent the effects of dexamethasone on clonidine-induced hypoactivity. The glucocorticoid receptor antagonist RU38486 administered centrally at the dose of 1 ng/mouse did not change the effects of clonidine, whereas it was able to block the effects of dexamethasone on clonidine-induced locomotor hypoactivity.

These results suggest that the effects of dexamethasone on clonidine-induced locomotor hypoactivity depend on the stimulating effects that dexamethasone exerts on the protein synthesis via the glucocorticoid receptor in the brain.

Clonidine is mainly considered to be an α_2-adrenoceptor agonist that, besides having a hypotensive action, induces anti-nociception and hypoactivity. Clonidine reduces the response to pain induced by a wide variety of stimuli including chemical (Skingle et al 1982; Luttinger et al 1984), heat (Lin et al 1980), pressure (Fielding et al 1978) and electrical stimuli (Paalzow & Paalzow 1976). The site of clonidine action is the central nervous system (Drew 1976; Drew et al 1979; Skingle et al 1982) where clonidine anti-nociception and locomotor hypoactivity are mediated by α_2-adrenoceptors (Heal et al 1989). Clonidine-induced anti-nociception and locomotor hypoactivity are antagonized by the α_2-adrenoceptor antagonist yohimbine (Paalzow & Paalzow 1976; Drew et al 1979; Rusterholtz et al 1980; Luttinger et al 1984).

We recently found that dexamethasone pretreatment in low doses reduced clonidine anti-nociception in mice (Pieretti et al 1995); this positive result further encouraged us to investigate the effects of dexamethasone on another behavioural effect exerted by clonidine in mice, locomotor hypoactivity.

In this study we have examined the possibility that peripheral or central dexamethasone administration could influence locomotor hypoactivity induced by clonidine in mice. Because in previous studies it has been demonstrated that dexamethasone exerts its effects after some delay (Tsurufuji et al 1979) and that dexamethasone may exert different effects when administered at low and high doses (Capasso et al 1991) we administered dexamethasone at various time intervals and at various doses before clonidine administration. We also investigated the influence of the glucocorticoid receptor antagonist RU38486 (Proulx-Terland et al 1982) on the effects induced by clonidine in animals pre-treated with dexamethasone. Finally, because it has been reported that cycloheximide prevented some of the effects of dexamethasone (Holaday et al 1978; Pieretti et al 1994), we performed some experiments to investigate whether the effects of dexamethasone may be prevented by cycloheximide pretreatment.

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Materials and Methods

Animals

Male Swiss mice (20–25 g) were supplied by Charles River (Italy). The animals were housed in colony cages (10 mice each) under standard lighting (lights on from 0700 to 1900 h), temperature (22 ± 1°C) and room humidity (60 ± 10%) conditions for at least 1 week before the experimental sessions. Food and water were freely available.

Locomotor activity

Locomotor activity was recorded in an activity cage (Basile, Milan) using a modification of a method previously reported (Capasso et al 1991). The animals were placed in the cage for at least 30 min for acclimatization before receiving injections of drugs. Temperature, sound and light conditions were maintained uniform during the course of the experiments, which lasted 2 h. Measurements were performed at 20-min intervals and cumulative counts were recorded (Capasso et al 1991).

Experimental procedure

On the day of testing all drugs used in the experimental sessions were dissolved in saline for intraperitoneal (i.p.) administration and in distilled water for intracerebroventricular (i.c.v.) administration. Drugs were injected in a volume of 10 mL kg^{-1}, intraperitoneally or 10 mL/mouse, intracerebroventricularly.

Dexamethasone (Sigma Chemical, USA) or RU38486 (Roussel-Uclaf, France) or cycloheximide (Sigma Chemical) were injected before clonidine (0·05 mg kg^{-1}, i.p.) according to the experimental schedule: dexamethasone (1 mg kg^{-1}, i.p.) 15 min, 30 min or 1 h before clonidine; dexamethasone (0·1 and 10 mg kg^{-1}, i.p.) 30 min before clonidine; dexamethasone (10 ng/mouse, i.c.v.) 30 min before clonidine; RU38486 (1 ng/mouse, i.c.v.) 15 min before clonidine in animals treated with dexamethasone (1 mg kg^{-1}, i.p.) 30 min before clonidine; cycloheximide (10 mg kg^{-1}, i.p.) 1·5 h before dexamethasone (1 mg kg^{-1}, i.p.) and 2 h before clonidine (0·05 mg kg^{-1}, i.p.).

Data were statistically analysed by one-way analysis of variance followed by multiple Newman-Keuls tests.

Results

Clonidine administered at doses of 0·05, 0·1 and 0·5 mg kg^{-1} produced a marked reduction in locomotor activity (Fig. 1). Dexamethasone administered intraperitoneally at a dose of 0·1, 1·0 and 10 mg kg^{-1} or at a dose of 10 ng intracerebroventricularly, or RU38486 administered intracerebroventricularly, or cycloheximide at a dose of 10 mg kg^{-1} did not change locomotor activity of mice (data not shown). Because no differences were observed between the effects of the three doses of clonidine, dexamethasone was administered to the animals treated with the lowest dose of clonidine (0·05 mg kg^{-1}). In the first series of experiments dexamethasone was administered at a dose of 1 mg kg^{-1} 15 min, 30 min or 1 h before clonidine. When dexamethasone was injected 15 min before clonidine no significant changes were observed in the locomotor hypoactivity induced by clonidine (Fig. 2). In contrast, dexamethasone administered 30 min or 1 h before clonidine significantly reduced this hypoactivity (Fig. 3). Dexamethasone administered at a dose of 0·1 mg kg^{-1} 30 min before clonidine did not change the locomotor hypoactivity induced by clonidine; at a dose of 10 mg kg^{-1}, however, it was able to induce a marked reduction of clonidine-induced locomotor hypoactivity that was higher in comparison with those observed with a dose of 1 mg kg^{-1} (Fig. 3). Dexamethasone administered intracerebroventricularly at a dose of 10 ng 30 min before clonidine was also able to reduce clonidine-induced locomotor hypoactivity (Fig. 3).

The effect induced by RU38486 and cycloheximide in animals pre-treated with dexamethasone is shown in Fig. 4. Administration of RU-38486 or cycloheximide did not change clonidine-induced locomotor hypoactivity (data not shown), but they were able to prevent dexamethasone effects on the reduction of locomotor activity induced by clonidine.

Discussion

These data indicate that dexamethasone reduced locomotor hypoactivity induced by clonidine in mice. The dexamethasone exerted its maximum effects 30–60 min after administration, suggesting that protein synthesis may be involved in the reduction of clonidine hypoactivity. Because glucocorticoids are known to exert many of their effects by stimulation of protein synthesis by an intracellular receptor mechanism (Thompson & Lippman 1974), dexamethasone was injected after administration of the protein synthesis inhibitor cycloheximide to test

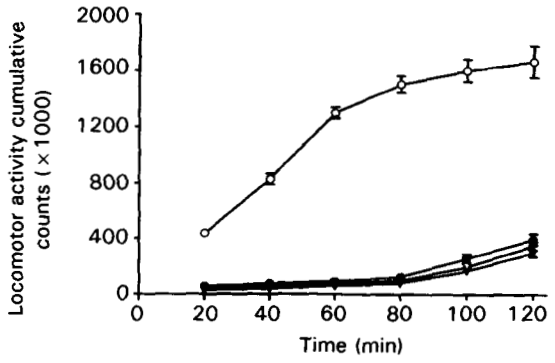

FIG. 1. Effects induced by saline and clonidine administered intraperitoneally (0·05, 0·1, 0·5 mg kg^{-1}) on the locomotor activity of mice. Note that clonidine induced a marked reduction in locomotor activity and that there is no significant difference between the effects of the three clonidine doses. Results are mean ± s.e.m. of cumulative counts recorded every 20 min for 2 h (n = 6). Saline (○); clonidine 0·05 mg (●); clonidine 0·1 mg (▽); clonidine 0·5 mg (▼).

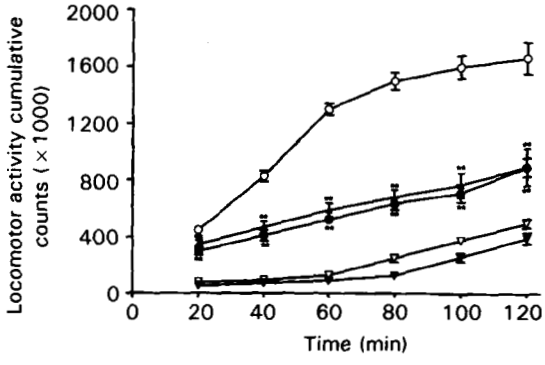

FIG. 2. Effect induced by dexamethasone (1 mg kg^{-1}, i.p.) on the reduction of the locomotor activity induced by clonidine (0·05 mg kg^{-1}, i.p.). Dexamethasone was administered 15 (▽), 30 (●) or 60 (△) min before clonidine. Results are mean ± s.e.m. of the cumulative counts recorded every 20 min for 2 h after clonidine administration. $^{**}P < 0·01$ compared with clonidine alone (▼). The effect of saline alone is also shown (○) (n = 6).

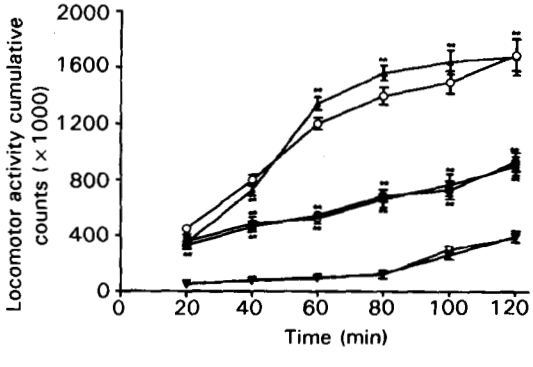

FIG. 3. Effect induced by dexamethasone (0·1 (▽); 1 (●); and 10 mg kg^{-1}, i.p. (▲) or 10 ng/mouse, i.c.v. (□) on the reduction of the locomotor activity induced by clonidine (0·05 mg kg^{-1}, i.p.). Dexamethasone was administered 30 min before clonidine. Results are mean ± s.e.m. of the cumulative counts recorded every 20 min for 2 h after clonidine administration. $^{**}P < 0·01$ compared with clonidine alone (▼). The effect of saline alone is also shown (○) (n = 6).

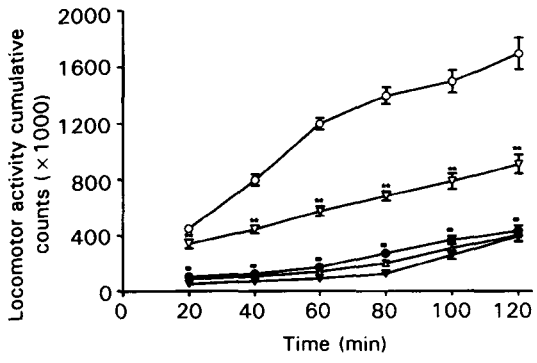

FIG. 4. Effects induced by RU-38486 (1 ng/mouse, i.c.v.) or cycloheximide (10 mg kg^{-1}, i.p) on the reduction by dexamethasone (1 mg kg^{-1}, i.p.) of the locomotor hypoactivity induced by clonidine (0·05 mg kg^{-1}, i.p.). Dexamethasone was administered 30 min before clonidine (▽); RU-38486 was administered 15 min before clonidine (●) and cycloheximide was administered 1·5 h before dexamethasone (△) and 2 h before clonidine. Results are mean ± s.e.m. of the cumulative counts recorded every 20 min for 2 h after clonidine administration. $^{**}P < 0.01$ compared with clonidine alone (▼); $^{\circ\circ}P < 0.01$ compared with dexamethasone + clonidine (▽) (n = 6). ○, Saline control.

whether in these experiments also protein synthesis was involved in the effects of dexamethasone. As expected, cycloheximide blocked the capacity of dexamethasone to reduce clonidine-induced hypoactivity. Some authors have suggested that glucocorticoid-induced reduction of the opioid effects might be mediated through an alteration in drug metabolism by the liver, presumably by enhancement of drug metabolism which therefore results in lower concentrations of the drug, thereby reducing its effect. Holaday et al (1978) reported a greater concentration of morphine in the blood and brains of adrenalectomized mice compared with sham control mice, and showed that dexamethasone treatment in the adrenalectomized animals restored morphine levels to those observed in sham control mice. They furthermore reported that dexamethasone-induced effects were reversed by cycloheximide pretreatment and that dexamethasone also reduced morphine levels in the blood and brain of sham control mice, indicating that glucocorticoid might induce an overall opiate-metabolizing activity. In our study we cannot exclude the possibility that the reduction of the effects of clonidine in animals pre-treated with dexamethasone might occur also by alteration of clonidine metabolism.

The results of our study suggest another hypothesis. Dexamethasone was able to reduce animals' sensitivity to clonidine even when dexamethasone was injected intracerebroventricularly at a low dose. In the nociceptive assays the effects induced by dexamethasone administered peripherally were, moreover, similar to those observed after central administration, suggesting a central site in the action of dexamethasone. In our experiments the effects of dexamethasone were, furthermore, prevented by central RU38486 administration.

The brain contains two types of receptor for corticosteroids, type-1 and type-2 (De Kloet & Reul 1987). Type-1 receptors are localized in the neurons of the hippocampus, lateral septum and amygdala, and resemble the kidney mineralocorticoid receptors. Type-1 receptors display the greatest affinity for corticosterone and aldosterone, but do not bind synthetic glucocorticoids (Reul & De Kloet 1985). Type-2 receptors are present both in neurons and in glial cells, and show widespread localization in the brain (Reul & De Kloet 1986). These receptors have greater affinity for synthetic glucocorticoids such as dexamethasone than does corticosterone (Reul & De Kloet 1986) and mediate the increase in the synthesis of some proteins that occur in the brain after glucocorticoid treatment (Schlatter & Dokas 1988). RU-38486 antagonizes dexamethasone at type-2 receptor binding sites (Moguilewski & Philbert 1984), blocks the inhibitory action exerted by dexamethasone on the pituitary-adrenal axis (Gaillard et al 1984), inhibits bethamethasone-induced vasocostriction (Gaillard et al 1985), and reverses the anti-inflammatory effects of dexamethasone (Peers et al 1988). All these findings suggest that in our experiments dexamethasone reduces clonidine-induced hypoactivity through stimulation of protein synthesis via type-2 glucocorticoid receptors in the brain.

Some reports indicate that corticosteroids may modulate peripheral and central binding to α_2-adrenoceptors, but this is still a matter for debate. Some reports have indicated that chronic corticosteroid treatment reduces the binding of clonidine in the brain cortex of rats (Szentendrei & Fekete 1990) and that some hypothalamic nuclei exhibit a diurnal variation of [^{3}H]*p*-aminoclonidine binding, with a significant decline in binding when plasma corticosterone levels are high (Jhanwar-Uniyal et al 1986). Maeda et al (1983), on the other hand, have studied changes in [^{3}H]clonidine binding in the vas deferens of rats treated with reserpine and found that the decrease in [^{3}H]clonidine binding was prevented by addition of hydrocortisone or dexamethasone to the culture medium. They also reported that the effect of glucocorticoid was blocked by the inhibitors of protein synthesis cycloheximide and puromicin. Adrenalectomy specifically reduced the binding of [^{3}H]*p*-aminoclonidine to the α_2-receptors in the hypothalamic paraventricular nucleus, and this down-regulation was reversed by corticosterone replacement (Jhanwar-Uniyal & Leibowitz 1985). These findings suggest the possibility that the effects of dexamethasone on clonidine depend on the possible influence that dexamethasone exerts on α_2-receptor binding properties.

Our study provides evidence that dexamethasone reduces some of the pharmacological effects induced by an α_2-stimulating agent, and that the interference of dexamethasone with clonidine-induced behavioural effects may also clarify the influence of steroids on the monoaminergic system.

References

Capasso, A., Di Giannuario, A., Loizzo, A., Pieretti, S., Sorrentino, L. (1991) Dexamethasone induces biphasic effect on morphine hypermotility in mice: a dose-related phenomenon. Life Sci. 49: 1411–1418

De Kloet, E. R., Reul, J. M. H. M. (1987) Feedback action and tonic influence of corticosteroids on brain function: a concept arising from the heterogeneity of brain receptor systems. Psychoneuroendocrinology 12: 83–87

Drew, G. M. (1976) Effects of α-adrenoceptor agonist and antagonist at pre- and post-synaptically located α-adrenoceptors. Eur. J. Pharmacol. 36: 313–320

Drew, G. M., Gower, A. J., Marriott, A. (1979) α_2-Adrenoceptors mediate clonidine-induced sedation in the rat. Br. J. Pharmacol. 67: 133–141

Fielding, S., Winkler, M., Hyner, M., Szewczak, W. J., Norick, W. J., Lal, H. (1978) A comparison of clonidine with morphine for antinuciceptive and withdrawal actions. Pharmacol. Exp. Ther. 207: 899–903

Gaillard, R. C., Riondel, A., Muller, A. F., Herrman, W., Baulieu, E. E. (1984) RU 486: a steroid with antiglucocorticosteroid activity that only disinhibits the human pituitary – adrenal system at a specific time of day. Proc. Natl. Acad. Sci. USA 81: 3879–3882

Gaillard, R. C., Poffet, D., Riondel, A. M., Saurat, J. H. (1985) RU 486 inhibits peripheral effects of glucocorticoids in humans. J. Clin. Endocrinol. Metab. 61: 1009–1015

Heal, D. J., Prow, M. R., Buckett, W. R. (1989) Clonidine-induced hypoactivity and mydriasis in mice are respectively mediated via pre- and post-synaptic α-adrenoceptor in the brain. Eur. J. Pharmacol. 170: 19–28

Holaday, J. W., Law, P. Y., Loh, H. H., Li, C. H. (1978) Adrenal steroids indirectly modulated morphine and β-endorphin effects. J. Pharmacol. Exp. Ther. 208: 176–180

Jhanwar-Uniyal, M., Leibowitz, S. F. (1985) Impact of circulating corticosterone on α_1- and α_2-noradrenergic receptors in discrete brain areas. Brain Res. 368: 404–410

Jhanwar-Uniyal, M., Roland, R. C., Leibowitz, S. F. (1986) Diurnal rhythm of α_2-noradrenergic receptors in the paraventricular nucleus and other brain areas: relation to circulating corticosterone and feeding behaviour. Life Sci. 38: 473–482

Lin, M. T., Chi, M. L., Chandra, A., Tsay, B. L. (1980) Serotoninergic mechanisms of beta-endorphin- and clonidine-induced analgesia in rats. Pharmacology 20: 323–328

Luttinger, D., Ferrari, R., Perrone, M. H., Haubrich, D. R. (1984) Pharmacological analysis of α_2 adrenergic mechanisms in nociception and ataxia. J. Pharmacol. Exp. Ther. 232: 883–889

Maeda, H., Watanabe, Y., Lai, R. T., Yoshida, H. (1983) Effect of glucocorticoids of α_2 adrenoceptor in vas deferens of reserpinized rat in organ culture. Life Sci. 33: 39–46

Moguilewski, M., Philibert, D. (1984) RU 38486: potent antiglucocorticoid activity correlated with strong binding to the cytosolic glucocorticoid receptor followed by an impaired activation. J. Steroid Biochem. 20: 271–276

Paalzow, G., Paalzow, L. (1976) Clonidine antinociceptive activity: effects of drugs influencing central monoaminergic and cholinergic mechanisms in the rat. Naunyn-Schmiedebergs Arch. Pharmacol. 292: 119–125

Peers, S. M., Moon, D., Flower, R. J. (1988) Reversal of the anti-inflammatory effects of dexamethasone by the glucocorticoid antagonist Ru 38486. Biochem. Pharmacol. 37: 556–559

Pieretti, S., Di Giannuario, A., Domenici, M. R., Capasso, A., Sorrentino, L., Loizzo, A. (1994) Dexamethasone-induced selective inhibition of the central μ opioid receptor: functional in vivo and in vitro evidence in rodents. Br. J. Pharmacol. 113: 1416–1422

Pieretti, S., Di Giannuario, A., Sorrentino, L., Capasso, A., Loizzo, A. (1995) Dexamethasone reduced clonidine-induced antinociception in mice. Pharmacol. Res. 31 (Suppl.): 258

Proulx-Terland, L., Cotè, J., Philibert, D., Deraedt, R. (1982) Potent antiglucocorticoid activity of RU 38486 on ACTH secretion in vitro and in vivo in the rat. J. Steroid Biochem. 17: 66–69

Reul, J. M. H. M., De Kloet E. R. (1985) Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinology 117: 2505–2509

Reul, J. M. H. M., De Kloet, E. R. (1986) Anatomical resolution of two types of corticosterone receptor sites in rat brain with in vitro autoradiography and computerized image analysis. J. Steroid Biochem. 24: 269–272

Rusterholtz, D. B., Dryer, S. E., Long, J. P., Barfnecht, C. F., Mott, J. (1980) Sedative and analgesic actions of methylated 2-aminotetralins: involvement of α_1 and α_2 adrenoceptors. Eur. J. Pharmacol. 65: 201–211

Schlatter, L. K. Dokas, L. A. (1988) Receptor specificity of a glucocorticoid – and stress-induced hippocampal protein. J. Neurosci. 9: 1134–1138

Skingle, M., Hayes, A. G., Tyers, M. B. (1982) Antinociceptive activity of clonidine in the mouse, rat and dog. Life Sci. 31: 1123–1132

Szentendrei, T., Fekete, M. I. K. (1990) Modulation of rat brain cortical α_2-adrenoceptor by treatment with hydrocortisone for 10 days. J. Neurochem. 54: 1852–1860

Thompson, E. B., Lippman, M. E. (1974) Mechanism of action of glucocorticoids. Metabolism 23: 159–161

Tsurufuji, I., Sugio, K., Takemasa, F. (1979) The role of the glucocorticoid receptor and gene expression in the anti-inflammatory action of dexamethasone. Nature 280: 408-410