Inhibitory effect of clonidine on ketamine-induced norepinephrine release from the medial prefrontal cortex in rats


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We have investigated the effect of clonidine on ketamine-induced norepinephrine release from the medial prefrontal cortex in rats using microdialysis. Twenty-one male Wistar rats weighing 200-300 g were allocated randomly to one of four groups: i.p. injection of ketamine 100 mg kg\(^{-1}\) with clonidine 0 (saline: group C0, \(n=6\)), 3 (group C3, \(n=5\)), 30 (group C30, \(n=5\)) and 300 \(\mu g\) kg\(^{-1}\) (group C300, \(n=5\)). As reported previously, ketamine increases norepinephrine release. In groups C0 and C3, marked increases in norepinephrine release were observed with maximum values of mean 483 (SEM 55)% and 412 (53)% compared with basal values, respectively. Although significant increases in norepinephrine release were also observed (276 (43)% in group C30, they were significantly lower than those in groups C0 and C3 (\(P<0.01\) and \(P<0.05\), respectively). In group C300, there was a significant reduction in norepinephrine release (62 (13)% compared with basal and the three other groups (\(P<0.01\)). This inhibitory effect of clonidine on norepinephrine may be related to reduction in undesirable emergence reactions after ketamine anaesthesia.

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Clonidine is an antihypertensive agent which is known to reduce central sympathetic outflow via central \(\alpha_2\) adrenoceptors. Central mediated sympatholysis induces sedation and perioperative haemodynamic stability. Therefore, \(\alpha_2\) agonists are used clinically for premedication.\(^1\) Clonidine penetrates the central nervous system (CNS) easily and one of the principal sites of action is thought to be the locus coeruleus which contains the largest clusters of noradrenergic neurones in the CNS.\(^2\)

Recently, we showed that ketamine markedly increased norepinephrine release from the medial prefrontal cortex in the rat using microdialysis.\(^3\) This suggests that ketamine may stimulate neurones in the locus coeruleus as noradrenergic neurones in the medial prefrontal cortex are derived mainly from the locus coeruleus.\(^3\)

The interaction between ketamine and clonidine in the control of norepinephrine release from the medial prefrontal cortex remains to be elucidated. In this study, we determined the effect of clonidine on ketamine-induced norepinephrine release from this brain region in the rat using microdialysis.

Methods and results

The study was approved by the Animal Care Committee of the University of Hirosaki School of Medicine. We studied 21 male Wistar rats weighing 200–300 g. A stainless guide cannula was implanted into the left medial prefrontal cortex with the following co-ordinates (A: 3.3, L:0.4, V: 2.0 mm) in relation to the bregma, according to the atlas of Paxinos (see Kubota and colleagues\(^3\)), under pentobarbital anaesthesia (50 mg kg\(^{-1}\) i.p.). On the day before the experiment, a microdialysis probe (A:1-4-03, Eicom, Kyto, Japan) was inserted into the guide cannula. The tip of the probe extended 3.0 mm beyond the tip of the guide to reach the medial prefrontal cortex. Artificial cerebrospinal fluid (composed of mmol litre\(^{-1}\): NaCl 128; KCl 2.6; CaCl\(_2\) 1.3; MgCl\(_2\) 0.9; NaHCO\(_3\) 20; Na\(_2\)HPO\(_4\) 1.3; and pargyline 1.0) was perfused through the dialysis probe at a rate of 1.0 \(\mu\)l ml\(^{-1}\).

Rats were allocated randomly to one of four groups: simultaneous i.p. injection of ketamine 100 mg kg\(^{-1}\) with clonidine 0 (saline: group C0, \(n=6\)), 3 (group C3, \(n=5\)), 30 (group C30, \(n=5\)) and 300 \(\mu g\) kg\(^{-1}\) (group C300, \(n=5\)). The injection volume did not differ between groups (0.1 ml kg\(^{-1}\)). Clonidine (clonidine hydrochloride, Wako Pure Chemical Industries Ltd, Osaka, Japan) and ketamine (Warner-Lambert, Tokyo, Japan) were dissolved in 0.9% saline.

After equilibration, samples of dialysate were collected
every 10 min. Three control samples were obtained before i.p. injection and then over 120 min after injection (total 15 samples per rat). They were injected automatically into a high-performance liquid chromatograph equipped with an electrochemical detector (ECD300, Eicom, Kyoto Japan) every 10 min, as described previously. Briefly, the mobile phase was phosphate buffer 0.1 mol litre⁻¹ containing 5% methanol and the oxidation potential of the graphite electrode was set at +0.40 V against an Ag–AgCl reference electrode. The flow rate of the mobile phase was 220 µl min⁻¹ onto the ODS-C18 reverse-phase column (Eicom, Kyoto, Japan). The detection limit of the assay was 125 fg 10 µl⁻¹.

Changes in norepinephrine release are expressed as percentage of basal release (mean value of the three samples before i.p. drug administration = 100%). To compare norepinephrine release between groups, area under the curve of percentage norepinephrine release from 0 to 120 min after i.p. injection of drug was measured (GraphPad Prism 1.0). All data are expressed as mean (SEM). Repeated measures ANOVA and one-way ANOVA followed by Fisher’s PLSD were used for intra-group and inter-group comparisons, respectively. P<0.05 was considered significant.

As reported previously, ketamine increased norepinephrine release (Fig. 1A). In groups CO, C3 and C30, from 20 min after i.p. administration of ketamine with clonidine, norepinephrine release increased significantly, with maximum increases of 483 (55)%, 412 (53)% and 276 (43)% respectively (Fig. 1A). However, in group C300, norepinephrine release decreased significantly to 62 (13)% of basal. This reduction in ketamine-stimulated norepinephrine release by clonidine was dose-dependent (Fig. 1B).

**Comments**

We found that clonidine inhibited ketamine-induced norepinephrine release from the medial prefrontal cortex in a dose-dependent manner. Our previous study suggested that ketamine may activate neurones in the locus coeruleus to increase norepinephrine release from the medial prefrontal cortex. Moreover, clonidine has been reported to hyperpolarize the locus coeruleus and reduce neuronal firing rate. As there are α₂ adrenoceptors in the terminals of noradrenergic neurones, clonidine may suppress the activity of not only the locus coeruleus but also noradrenergic neurones in the cortex to reduce ketamine-induced norepinephrine release. As Hancock and Stamford reported recently that ketamine released dopamine directly from terminals of dopaminergic neurones in the nucleus accumbens, it is possible that norepinephrine release may also be released directly from terminals by ketamine. However, the interaction of ketamine with the dopaminergic system in the brain is complex. Ketamine may not affect nigrostriatal dopaminergic neurones, although ketamine activates mesolimbic dopaminergic neurones (see Hancock and Stamford). In addition, we have observed that ketamine does not increase basal or K⁺-evoked norepinephrine release from the cortex (K. Hirota and M. Kudo, 1999, unpublished observation). Therefore, norepinephrine release from the medial prefrontal cortex by ketamine may reflect locus coeruleus activity.

Ketamine produces potent analgesic and hypnotic actions without depression of the cardiovascular or respiratory systems. However, after ketamine anaesthesia, undesirable post-anaesthesia emergence reactions such as vivid dreams, hallucinations and agitation are also reported. This is the reason for the limited clinical use of ketamine as a sole anaesthetic agent.

α₂-Adrenoceptor agonists have been the subject of considerable attention in recent years because of their unique sedative, hypnotic and analgesic properties. In addition, undesirable central effects of ketamine may be prevented by α₂ agonists, as Levänen, Muffelman and Scheinin reported that premedication with dexmedetomidine, a highly selective α₂ agonist, attenuated not only post-anaesthetic delirium but also cardiostimulation caused by ketamine.
There have been no reports of the neuronal mechanisms of the inhibitory actions of clonidine on ketamine-induced emergence reactions. The noradrenergic system in the locus coeruleus–medial prefrontal cortex is involved in emotional behaviour. Our data may represent an explanation for these findings, although we did not evaluate behavioural changes in the rats.

In summary, we have demonstrated that clonidine inhibited ketamine-induced norepinephrine release from the medial prefrontal cortex in a dose-dependent manner. This effect may be involved in the mechanism of the inhibitory effect of clonidine on the undesirable emergence reactions after ketamine anaesthesia.

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