Brain Research 763 (1997) 281–284

Short communication

Effects of ketamine on dopamine metabolism during anesthesia in discrete brain regions in mice: comparison with the effects during the recovery and subanesthetic phases

Masahiro Irifune [a,d,*], Takeo Fukuda [b], Masahiro Nomoto [b], Tomoaki Sato [a], Yoshiko Kamata [a], Takashige Nishikawa [a], Wataru Mietani [c], Kozo Yokoyama [c], Kazuna Sugiyama [c], Michio Kawahara [d]

[a] *Department of Pharmacology, Kagoshima University Dental School, Kagoshima 890, Japan*
[b] *Department of Pharmacology, Kagoshima University Medical School, Kagoshima 890, Japan*
[c] *Department of Anesthesiology, Kagoshima University Dental Hospital, Kagoshima 890, Japan*
[d] *Department of Anesthesiology, Hiroshima University Dental Hospital, 1-2-3 Kasumi, Minami-ku, Hiroshima 734, Japan*

Accepted 22 April 1997

Abstract

The effects of ketamine on the levels of dopamine (DA), norepinephrine (NE), 5-hydroxytryptamine (5-HT, serotonin) and their metabolites were examined in discrete brain regions in mice. A high dose of ketamine (150 mg/kg, i.p.) did not change DA metabolism in the frontal cortex, nucleus accumbens, striatum and hippocampus, but did decrease it in the brainstem during anesthesia. In contrast, during recovery from the ketamine anesthesia, the high dose increased the level of homovanillic acid (HVA) in all brain regions. A low subanesthetic dose of ketamine (30 mg/kg, i.p.) increased the concentrations of both 3,4-dihydroxyphenylacetic acid (DOPAC) and HVA only in the nucleus accumbens. The DA level was not affected by any ketamine treatment. During ketamine anesthesia, the content of 3-methoxy-4-hydroxy-phenylglycol (MHPG) was decreased in the brainstem, whereas during recovery from anesthesia, the MHPG level was increased in the frontal cortex, nucleus accumbens and brainstem. The NE content was not altered in any region by ketamine treatment. The concentration of 5-hydroxyindoleacetic acid (5-HIAA) was reduced in the frontal cortex, striatum, hippocampus and brainstem during ketamine anesthesia. The 5-HT level was unaltered in all regions except the brainstem where it was reduced. In contrast, after anesthesia, the concentrations of both 5-HT and 5-HIAA were increased in the striatum. During the subanesthetic phase, however, the levels of NE, 5-HT and their metabolites were unchanged. These neurochemical results are consistent with the electrophysiological findings that a high dose of ketamine does not change the basal firing rates of nigrostriatal DA neurons during anesthesia, while low subanesthetic doses significantly increase those of ventral tegmental DA neurons.

Keywords: 3,4-Dihydroxyphenylacetic acid; Homovanillic acid; Norepinephrine; 3-Methoxy-4-hydroxy-phenylglycol; 5-Hydroxytryptamine; 5-Hydroxyindoleacetic acid; Striatum; Nucleus accumbens

It has been demonstrated in rodent brain that the short-term accumulation of the major dopamine (DA) metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in the striatum provides an accurate reflection of activity in the dopaminergic neurons of the nigrostriatal pathway. A cessation of impulse flow after the placement of acute lesions in the nigrostriatal pathway leads to a rapid decrease in striatal DOPAC and HVA. Conversely, electrical stimulation of the nigrostriatal pathway results in a frequency-dependent increase in DOPAC and HVA within the striatum. Thus, there appears to be an excellent correlation between changes in impulse flow in dopaminergic neurons and changes in DOPAC and HVA [1,8]. In electrophysiological studies, general anesthetics such as halothane, pentobarbital and chloral hydrate have been reported to increase impulse flow in DA neurons of the nigrostriatal pathway in rats [6,8]. In neurochemical studies, it has consistently been shown that these anesthetics increase the metabolism and turnover of DA in the striatum of rats [8,9].

In contrast to these general anesthetics, it has recently

* Corresponding author at address 'd'. Fax: +81 (82) 257-5779.

been reported electrophysiologically that the dissociative anesthetic ketamine does not change the basal firing rates of nigrostriatal DA neurons during the anesthesia [4]. Biochemically, although many reports have examined the effects of ketamine on DA metabolism, only two studies have investigated DA metabolism during ketamine anesthesia, and these two studies in rats gave inconsistent results: ketamine (100 mg/kg, i.p.) was reported to increase the striatal HVA level at 15 min and 60 min after administration [10], while the same dose was found not to change striatal levels of DOPAC and HVA for at least 6 h after injection [5]. To explore this discrepancy further, the effects of ketamine on DA metabolism during anesthesia were examined in this study not only in the striatum, but also in the frontal cortex, nucleus accumbens, hippocampus and brainstem, in mice. Furthermore, the effects during the ketamine anesthesia were compared with those during the recovery and subanesthetic phases, and the contents of norepinephrine (NE), 5-hydroxytryptamine (5-HT, serotonin) and their metabolites in these brain areas were measured.

Adult male ddY mice (37–49 g) were housed in groups with free access to food and water under conditions of controlled temperature (23 ± 1°C), humidity (50 ± 5%) and lighting (dark period 1900–0700 h). They were sacrificed by decapitation at various times after the i.p. administration of ketamine (30 or 150 mg/kg) or saline. The frontal cortex, nucleus accumbens, striatum, hippocampus and brainstem were immediately dissected and kept at −40°C until assayed. Monoamines and their metabolites were determined by high-performance liquid chromatography (HPLC) with electrochemical detection (ECD) according to a method previously described by this laboratory [3]. The data were analyzed by one-way analysis of variance (ANOVA) with the least significant difference (LSD) test or Student's *t*-test. The results in the text and tables are expressed as the mean ± S.E.M.

In a preliminary study, the effect of ketamine (30 or

Table 1
Effects of ketamine on levels of dopamine (DA) and its metabolites in discrete brain regions in mice

Region and dose (mg/kg)	Time (min)	DA (ng/g tissue)	DOPAC (ng/g tissue)	HVA (ng/g tissue)
Frontal cortex				
Control	10	247 ± 113	128 ± 15	186 ± 12
150	10	238 ± 72	136 ± 18	221 ± 17
150	120	169 ± 31	123 ± 14	290 ± 14 [a]
Control	10	145 ± 35	100 ± 11	177 ± 9
30	10	155 ± 49	99 ± 15	169 ± 10
Nucleus accumbens				
Control	10	7809 ± 479	1516 ± 62	878 ± 53
150	10	7711 ± 687	1404 ± 61	926 ± 36
150	120	7610 ± 661	1610 ± 125	1411 ± 59 [a]
Control	10	6502 ± 184	1261 ± 39	801 ± 27
30	10	6776 ± 325	1495 ± 46 [a]	934 ± 14 [a]
Striatum				
Control	10	14492 ± 534	2030 ± 108	1453 ± 50
150	10	13870 ± 435	1848 ± 98	1516 ± 46
150	120	13401 ± 360	2131 ± 99	2273 ± 97 [a]
Control	10	15018 ± 701	1809 ± 90	1377 ± 48
30	10	14555 ± 743	1703 ± 116	1396 ± 43
Hippocampus				
Control	10	n.d.	n.d.	95 ± 4
150	10	n.d.	n.d.	91 ± 19
150	120	n.d.	n.d.	171 ± 10 [a]
Control	10	n.d.	n.d.	143 ± 38
30	10	n.d.	n.d.	139 ± 9
Brainstem				
Control	10	46 ± 5	61 ± 4	72 ± 11
150	10	42 ± 2	48 ± 2 [a]	51 ± 3
150	120	42 ± 2	57 ± 3	104 ± 4 [a]
Control	10	45 ± 2	55 ± 3	74 ± 6
30	10	52 ± 11	65 ± 7	83 ± 13

Mice were decapitated 10 min after i.p. administration of 30 mg/kg of ketamine and 10 or 120 min after 150 mg/kg of ketamine. Control animals were sacrificed 10 min after i.p. injection of saline. Values are mean ± S.E.M. ($n = 6–7$).

[a] $P < 0.01$ compared with control animals, one-way ANOVA with the least significant difference test or Student's *t*-test.

n.d. = not detectable.

150 mg/kg, i.p.) on the righting reflex was examined. At a dose of 150 mg/kg, ketamine produced complete loss of the righting reflex 3 min after the administration. The duration of this loss of the righting reflex was 25 ± 3 min ($n = 6$). Thereafter, the effect declined gradually but the mice did not recover a normal righting reflex until at least 60 min after the injection. In contrast, 30 mg/kg of ketamine induced no loss of the righting reflex, but did produce a slight impairment of the reflex; the animals righted themselves within 2 s but the hindlimb was raised. This peak effect occurred at 3 min postinjection, followed by a rapid decline; the mice returned to normal within 10 min ($n = 6$). Therefore, the metabolism of monoamines was determined 10 min after the administration of 150 mg/kg of ketamine (anesthetic phase), 120 min after 150 mg/kg (recovery phase) or 10 min after 30 mg/kg (subanesthetic phase).

Ten minutes after the injection of 150 mg/kg of ketamine (anesthetic phase), the levels of DOPAC and HVA were unchanged in all brain regions tested except the brainstem. In the brainstem, the concentration of DOPAC was decreased by 21%. In contrast, 120 min after the injection of the high dose (recovery phase), the concentration of HVA was increased by 44 to 80% in all regions. Ten minutes after the administration of 30 mg/kg of ketamine (subanesthetic phase), the only changes in DOPAC and HVA levels were increases of 19% and 17%, respectively, in the nucleus accumbens. The DA level was not modified in any brain region at any time following the injection of any dose of ketamine (Table 1).

The results of the ketamine-induced change in the levels of NE, 5-HT and their metabolites in different brain areas are summarized in Table 2. During ketamine anesthesia, the content of the major metabolite of NE, 3-methoxy-4-hydroxy-phenylglycol (MHPG), was decreased by 15% in the brainstem, whereas during the recovery phase, the MHPG level was increased in the frontal cortex, nucleus accumbens and brainstem by 58, 42 and 37%, respectively.

Table 2
Effects of ketamine on levels of norepinephrine (NE), 5-hydroxytryptamine (5-HT) and their metabolites in discrete brain regions in mice

Region, dose (mg/kg)	Time (min)	NE (ng/g tissue)	MHPG (ng/g tissue)	5-HT (ng/g tissue)	5-HIAA (ng/g tissue)
Frontal cortex					
Control	10	449 ± 6	65 ± 17	679 ± 25	326 ± 23
150	10	439 ± 13	53 ± 15	688 ± 30	270 ± 10 [b]
150	120	433 ± 20	103 ± 6 [a]	756 ± 31	357 ± 20
Control	10	441 ± 27	194 ± 40	686 ± 25	358 ± 16
30	10	445 ± 18	169 ± 44	655 ± 29	333 ± 12
Nucleus accumbens					
Control	10	900 ± 42	134 ± 10	1333 ± 86	664 ± 39
150	10	957 ± 47	131 ± 4	1333 ± 36	578 ± 42
150	120	1002 ± 84	190 ± 14 [b]	1488 ± 57	734 ± 45
Control	10	958 ± 80	135 ± 39	1281 ± 89	623 ± 37
30	10	837 ± 50	211 ± 62	1250 ± 18	614 ± 23
Striatum					
Control	10	295 ± 12	97 ± 4	686 ± 20	572 ± 19
150	10	308 ± 12	111 ± 7	677 ± 21	488 ± 20 [b]
150	120	331 ± 8	117 ± 7	757 ± 22 [a]	724 ± 28 [b]
Control	10	250 ± 11	97 ± 3	644 ± 16	482 ± 24
30	10	267 ± 3	82 ± 18	637 ± 14	491 ± 19
Hippocampus					
Control	10	600 ± 28	n.d.	525 ± 20	837 ± 64
150	10	625 ± 32	n.d.	604 ± 33	555 ± 131 [b]
150	120	623 ± 23	n.d.	637 ± 28 [a]	970 ± 63
Control	10	539 ± 44	n.d.	500 ± 20	659 ± 68
30	10	551 ± 11	n.d.	540 ± 35	740 ± 52
Brainstem					
Control	10	772 ± 35	117 ± 5	858 ± 77	807 ± 55
150	10	679 ± 26	100 ± 6 [a]	680 ± 36 [b]	514 ± 39 [b]
150	120	724 ± 27	160 ± 7 [b]	858 ± 19	795 ± 42
Control	10	726 ± 28	102 ± 7	738 ± 31	625 ± 40
30	10	706 ± 28	110 ± 6	752 ± 30	589 ± 20

Mice were decapitated 10 min after i.p. administration of 30 mg/kg of ketamine and 10 or 120 min after 150 mg/kg of ketamine. Control animals were sacrificed 10 min after i.p. injection of saline. Values are mean ± S.E.M. ($n = 6$–7).
[a] $P < 0.05$, [b] $P < 0.01$ compared with control animals, one-way ANOVA with the least significant difference test or Student's *t*-test.
n.d. = not detectable.

The NE content was not altered in any region by ketamine treatment.

The concentration of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), was reduced in the frontal cortex, striatum, hippocampus and brainstem by 17, 15, 34 and 36%, respectively, during ketamine anesthesia. The serotonin level was unaltered in all regions except the brainstem where serotonin was reduced by 21%. In contrast, after the anesthesia, the 5-HIAA concentration increased to 127% and the serotonin level increased to 110% in the striatum. During the subanesthetic phase, however, the levels of NE, serotonin and their metabolites were unchanged.

The present study showed that a high dose of ketamine (150 mg/kg, i.p.) did not change DA metabolism in the frontal cortex, nucleus accumbens, striatum and hippocampus during anesthesia, but did decrease it in the brainstem. In contrast, during recovery from ketamine anesthesia, the high dose increased the DA turnover in all brain regions. Moreover, a low subanesthetic dose of ketamine (30 mg/kg, i.p.) increased the metabolism only in the nucleus accumbens (Table 1). These results are consistent with the electrophysiological findings that a high dose of ketamine does not change the basal firing rates of nigrostriatal DA neurons in rats during the anesthesia [4], while low subanesthetic doses significantly increase those of ventral tegmental DA neurons, which originate in the ventral tegmental area and terminate mainly in the nucleus accumbens and the olfactory tubercle, in rats [2].

It is of practical interest that the effects of ketamine on DA metabolism differ in the anesthetic, the recovery and the subanesthetic phases, and in the various brain regions. It has been proposed that there are a number of ways in which drugs can alter impulse flow [1]. For example, (1) a drug can act directly on the nerve cell body, (2) it can act on other neurons, which then influence impulse flow in the neuron under study, (3) it can act at the autoreceptor, and (4) it can act at the postsynaptic receptor to cause stimulation or blockade, which then results in some sort of feedback influence on the presynaptic neuron. The precise nature of the mechanism of the ketamine action on DA metabolism remains unknown. However, it seems likely that ketamine acts on other excitatory and/or inhibitory neurons, which ultimately change or fail to change the impulse flow in the DA neurons. Ketamine has been reported to act on several receptors such as *N*-methyl-D-aspartate, muscarinic cholinergic and sigma receptors, in addition to monoaminergic neurons [7]. This mechanism may be supported by our findings that the effects of ketamine on NE and serotonin metabolism are different from those on DA metabolism (Tables 1 and 2).

Acknowledgements

The authors thank Yoshiteru Toriire for technical assistance.

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