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EFFECT OF ASPARTATE AND GLUTAMATE ON NOCICEPTION, CATALEPSY AND CORE TEMPERATURE IN RATS

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Abstract : Effects of excitatory aminoacids (EAAs) aspartate (ASP) and glutamate (GLU) in a low (50 ng, i.c.) and high dose (20 µg, i.c.), were studied on nociception, catalepsy and rectal temperature in albino rats. Both ASP and GLU altered the tail flick reaction time to thermal stimulation in a dose dependent manner, increasing it with low doses and reduced with high doses. Naloxone (10 µg, ic) antagonized the anti-nociceptive effect of EAAs while ketamine (10 µg, ic)-a NMDA receptor antagonist antagonized the hyperalgesic effect. These EAAs also antagonized catalepsy induced by haloperidol, chlorpromazine, trifluoperazine and morphine. Both ASP and GLU produced a hyperthermic response in all animals, including those in which hypothermia was induced by reserpine. These EAAs produced a comparable central modulatory effects on nociception, catalepsy and core temperature.

Key words : aspartate, analgesia, catalepsy, glutamate

INTRODUCTION

Aspartate (ASP) and glutamate (GLU) are the predominant excitatory neurotransmitters in the mammalian spinal cord (1) and various parts of the brain (2). It has been proposed that the sensory neurons subserved by these EAAs possess large diameter axons participating in monosynaptic reflex arcs and coursing within the dorsal columns (3). Recently however, data has been provided suggesting that EAAs may be released from small diameter primary afferents involved in nociception. Glutamate has also been shown to produce nociceptive effect in rat and mice (4, 5). On the other hand Martinez (6) reported that monosodium glutamate increases or decreases analgesic effects of morphine in rats depending upon the analgesic test used and on the gender. Raigordosky (7) demonstrated that injection of the n-methyl-D-aspartate into subarachnoid space of rats produces both analgesic and hyperalgesic effects. Many recent studies using *in vitro* preparations such as brain slices (2) and synaptosomes (8) have mainly concentrated on demonstrating neurotransmitter role of ASP and GLU in the brain. In view of these conflicting reports the present study was carried out to evaluate effects of the EAAs on nociception, catalepsy and rectal temperature in rats.

METHODS

1. Analgesia : In male albino rats (180–190 g) analgesia was evaluated using tail flick test

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to thermal stimulation by analgesiometer (9). The heat intensity was adjusted such that the rats had control (pre-drug) tail flick latencies of 3 to 4 sec. A 10 sec cutoff latency was used to prevent damage to tail. Intracisternal (i.c.) injections were given by a microsyringe over a period of 20 sec as described by Jaffers & Griffith (10). The initial (control) reaction time was recorded and animals were divided into 8 groups of 10 rats each. In groups I and II ASP and GLU (50 ng/rat, i.c., in a volume of 10 µl of normal saline) respectively were given immediately after initial tail flick testing. Animals of group III and IV were pretreated (15 min) with 10 µg naloxone/rat, ic along with ASP and GLU (50 ng, ic) respectively. High doses (20 µg/rat, ic) of ASP and GLU were given in animals of group V and VI. Ketamine (10 µg/rat, ic) was given 30 min before ic injections of ASP and GLU (20 µg/rat, ic) in animals of group VII and VIII and reaction time was noted at 30 min, 1 hr, 2 hr and 3 hr. Effects of these drugs were evaluated by using each animal as its own control.

Low (50 ng/rat) and high (20 µg/rat) dose of ASP and GLU was selected after a pilot study using different doses i.e. 25, 50, 100 ng/rat and 5, 10, 20 µg/rat respectively.

2. *Catalepsy:* Catalepsy was evaluated in albino rats (180–190 g) by placing the front limbs of the animal over a horizontal bar positioned 8 cm above the floor and measuring the time for which the animal maintained the posture. Scoring was done by the method of Balsara (11). Animals were divided into 4 groups of 30 rats each. Each group was further subdivided into 3 subgroups. In groups Ia, IIa IIIa and IVa, haloperidol (5 mg/kg), chlorpromazine (15 mg/kg), trifluoperazine (10 mg/kg) and morphine (15 mg/kg) respectively were given intraperitoneally to produce catalepsy. Groups Ib and Ic received haloperidol, groups IIb and IIc chlorpromazine, group IIIb and IIIc trifluoperazine and groups IVb and IVc received morphine respectively after pretreatment with ASP and GLU (20 µg/rat, i.c, 30 min before). Cataleptic score was recorded at 30 min, 1 hr, 2 hr and 3 hr.

3. *Rectal temperature:* Rectal temperature was measured in albino rats (180–190 g) at 30 min, 1 hr, 2 hr and 3 hr by a rectal thermometer. After recording control rectal temperature the animals were divided into 2 groups of 10 rats each. Group I received ASP 20 µg, ic while group II received GLU (20 µg/rat, ic). Predrug and post drug rectal temperature was compared.

In another series of experiments after recording control rectal temperature, reserpine (5 mg/kg, ip) were given to albino rats (n = 10). After 15 hr of reserpine administration animals were divided into 2 groups of 10 rats each. In groups I and II ASP and GLU (20 µg/rat, ic) respectively were given in a volume of 10 µl of saline. The student's 't' test was used for the statistical analysis of the results.

RESULTS

Analgesia - In low doses (50 ng/rat, ic) both ASP and GLU significantly ($P < 0.01$) increased the tail flick response in rats. The maximum antinociceptive response of ASP and GLU was observed after 30 min of intracisternal injection. The antinociceptive effect of ASP and GLU was antagonized by naloxone (10 µg/rat, ic). In high doses (20 µg/rat, ic) both ASP and GLU produced hyperalgesic effect along with some signs of excitatory behaviour i.e. increased hyperactivity to sound, motor activity and biting. This hyperalgesic effect of ASP and GLU was antagonized by ketamine 10 µg/rat, ic – an NMDA receptor antagonist (Table I).

TABLE I : Effect of low (50 ng/rat, ic) and high (20 µg/rat, ic) doses of ASP and GLU on reaction time to tail flick test in rats. Data represented as mean ± SE.

Groups	Reaction time (sec)				
	Control	*30 min*	*1 hr*	*2 hr*	*3 hr*
I ASP (50 ng)	3.35 ± 0.15	4.45 ± 0.16*	3.85 ± 0.13*	3.75 ± 0.20	3.40 ± 0.24
II GLU (50 ng)	3.20 ± 0.13	4.45 ± 0.17*	3.90 ± 0.15*	3.75 ± 0.21	3.25 ± 0.15
III NAL (10 µg)+ ASP	3.15 ± 0.07	3.35 ± 0.08	3.35 ± 0.10	3.25 ± 0.15	3.20 ± 0.10
IV NAL (10 µg)+ GLU	3.85 ± 0.10	4.05 ± 0.15	4.00 ± 0.10	3.90 ± 0.14	3.80 ± 0.12
V ASP (20 µg)	3.25 ± 0.12	2.65 ± 0.05*	2.75 ± 0.03*	2.95 ± 0.06*	3.15 ± 0.08
VI GLU (20 µg)	3.75 ± 0.20	3.10 ± 0.10*	3.15 ± 0.08*	3.50 ± 0.05	3.50 ± 0.06
VII KET (10 µg)+ ASP	3.40 ± 0.15	3.55 ± 0.07	3.55 ± 0.07	3.15 ± 0.08	3.00 ± 0.08
VIII KET (10 µg)+ GLU	3.95 ± 0.04	4.05 ± 0.15	4.15 ± 0.14	3.85 ± 0.07	3.75 ± 0.09

*P< 0.05 when compared with control.

TABLE II : Effect of intracisternal ASP and GLU (20 µg/rat) on catalepsy induced by haloperidol, chlorpromazine, trifluoperazine and morphine in rats. Data represented as mean cataleptic score ± SE.

Groups	Mean cataleptic score ± SE			
	After 30 min	*1 hr*	*2 hr*	*3 hr*
I (a) HALO	3.1 ± 0.23	3.6 ± 0.16	3.5 ± 0.16	2.3 ± 0.15
(b) ASP + HALO	1.8 ± 0.13*	1.8 ± 0.13*	1.4 ± 0.20*	0.5 ± 0.16*
(c) GLU + HALO	1.5 ± 0.16*	1.7 ± 0.15*	1.6 ± 0.15*	0.2 ± 0.13*
II (a) CPZ	3.2 ± 0.18*	3.3 ± 0.15	3.3 ± 0.15	2.5 ± 0.13
(b) ASP + CPZ	1.7 ± 0.20*	1.9 ± 0.21*	1.5 ± 0.19*	0.6 ± 0.16*
(c) GLU + CPZ	1.8 ± 0.21*	1.8 ± 0.21*	1.2 ± 0.12*	0.4 ± 0.16*
III (a) TRIF	3.5 ± 0.12	3.7 ± 0.15	3.7 ± 0.05	2.0 ± 0.24
(b) ASP + TRIF	1.4 ± 0.24*	1.5 ± 0.16*	1.4 ± 0.24*	0.6 ± 0.28*
(c) GLU + TRIF	1.6 ± 0.16*	1.7 ± 0.15*	1.3 ± 0.15*	0.8 ± 0.12*
IV (a) MORP	3.5 ± 0.12	3.7 ± 0.15	3.5 ± 0.12	1.6 ± 0.16
(b) ASP + MORP	0.3 ± 0.15*	0.3 ± 0.15*	0.2 ± 0.14*	0.0 ± 0.00*
(c) GLU + MORP	0.5 ± 0.16*	0.4 ± 0.16*	0.3 ± 0.15*	0.1 ± 0.00*

*P < 0.01 when compared to corresponding controls i.e. group Ia, IIa, IIIa and IVa.
HALO - Haloperidol, CPZ-chlorpromazine, TRIF - Trifluoperazine and MORP - morphine.

Catalepsy - Pretreatment with ASP and GLU (20 µg/rat, ic) significantly (P < 0.01) antagonized catalepsy induced by haloperidol chlorpromazine, trifluoperazine and morphine. The antagonistic effect of ASP and GLU was more marked in morphine induced catalepsy as compared to catalepsy produced by haloperidol, chlorpromazine and trifluoperazine (Table II).

Rectal temperature - Intracisternal injections of ASP and GLU (20 µg/rat, ic) produced a significant ($P < 0.01$) hyperthermic response in rats. The peak effects was observed after 30 min of ASP and GLU administration (Table III). Both ASP and GLU significantly antagonized hypothermic effect of reserpine (5 mg/kg, ip), and rather produced a hyperthemic response in reserpine pretreated (15 hr before) animals (Table IV).

TABLE III : Effect of intracisternal ASP and GLU (20 µg/rat) on rectal temperature. Data represented as mean ± SE.

	Groups	Rectal temperature °C				
		Control	*30 min*	*1 hr*	*2 hr*	*3 hr*
I	ASP	36.91 ± 0.07	37.44± 0.08*	37.27 ±0.07*	37.02± 0.06	36.98 ± 0.06
II	GLU	36.87 ± 0.06	37.54± 0.05*	37.36 ±0.09*	37.03± 0.08	36.84 ± 0.09

*P < 0.01 when compared to control.

TABLE IV : Effect of ASP and GLU (20 µg/rat, ic) on rectal temperature in reserpine (5 mg/kg, ip) hypothermic rats. Data represented as mean ± SE

Groups	Rectal temperature °C					
	Control	*After 15 hrs of reserpine*	*After 30 min*	*1 hr*	*2 hr*	*3 hr*
I	36.86 ± 0.02	35.35 ± 0.07**	36.39±0.07*	37.64±0.07*	37.51±0.08*	37.10±0.08*
II	36.90 ± 0.02	35.55 ± 0.10**	36.71±0.10*	37.68±0.02*	37.59±0.04*	37.27±0.11*

*P < 0.05 when compared to reserpine.
**P < 0.05 when compared to control.

DISCUSSION

The antinociceptive effect of ASP and GLU observed with low concentrations in this study may be due to actions of these excitatory aminoacids at the spinal level and partly on the endogenous opioid peptide system. Both ASP and GLU have been found in appreciable amounts in the spinal cord. A high GLU immunoreactivity has been reported to be located inside nerve terminals in the laminae I and II in the dorsal horn of the rat (12). Skilling et al (13) demonstrated for the first time in freely moving rats the *in vivo* release of ASP and GLU from dorsal horn of the spinal cord after putative nociceptive stimulation. It is possible that in low doses both ASP and GLU may affect descending tonic inhibitory system which is believed to be involved in antinociceptive information or these EAAs may activate endogenous segmental pain inhibitory mechanisms. Our findings are in agreement with Raigordosky (7) who also reported that in low concentrations intrathecal administration of excitatory amino acid n-methyl-D-aspartate activates antinociceptive system at the level of spinal cord. These workers further demonstrated that spinalization (removed tonic facilitation) does not attenuate but even enhances the analgesic

effect of n-methyl-D-aspartate indicating the spinal origin of its analgesic effect. However, the fact that naloxone was able to block the analgesic effect of ASP and GLU observed in the present study suggests involvement of endogenous opioid peptide system. Our contention is further supported by the findings of Martinez (6) who demonstrated that monosodium glutamate in immature rats produce analgesic effect by exerting effects on cells and fibers containing metenkephalin and beta endorphin. Therefore, it may be possible that ASP and GLU facilitate release of endogenous opioid peptides from neurons which may ultimately be responsible for the observed antinociceptive effect.

The nociceptive effect of ASP and GLU observed with high concentrations may be mediated via activation of NMDA receptors, possibly through the generation of nitric oxide synthesis (5). The hyperalgesic response and pain behaviour of both ASP and GLU was blocked by ketamine - noncompetitive NMDA receptor antagonist (14). This indicates involvement of NMDA receptor activation in nociceptive action.

Anticataleptic effect of ASP and GLU in catalepsy induced by haloperidol, chlorpromazine and trifluoperazine may be due to increased release of dopamine (DA) by ASP and GLU in the striatum. Our finding is supported by an *in vitro* study (15) in which L-glutamate produced a concentration dependent release of ($_3$H) - DA from slices of the nucleus accumbens, olfactory tubercle, caudate putamen and substantia nigra of rat by acting at a Mg^{++} sensitive NMDA receptor (15). Thus ASP and GLU antagonize the catalepsy by increasing the release of DA from nerve terminals in the rat striatum, while haloperidol, phenothiazines, chlopromazine and trifluoperazine are known to block DA receptor and decrease striatal DA concentrations (16). Morphine, and related narcotic analgesics increase turnover of DA indirecly, to produce extrapyramidal effects (16).

The significant hyperthermic response after intracisternal administration of both ASP and GLU in rats may be due to modulation of actions of norepinephrine. Our results are further supported by the observations that ASP and GLU significantly antagonised reserpine induced hypothermia in rats. Reserpine hypothermia appears to be related to the loss of norepinephrine from both central and peripheral nerve terminals by inhibition of uptake and storage (17). Thus it is possible that ASP and GLU act by increasing the availability of norepinephrine, possibly by increasing the release from nerve terminals. Our contention is further supported by a recent study of Navarro et al (18) who demonstrated that glutamate stimulates norepinephrine release in several areas of the rat brain. Intraischemic hypothermia has been reported to decrease the release of glutamate in cerebral infarcts (19) showing a reverse correlation between glutamate and hyperthermia.

In conclusion, this study suggests that the EAAs ASP and GLU caused a dose dependent effect on tail flick reaction time to thermal stimulation, increasing it with low dose while reduce it with high dose. Both ASP and GLU antagonize catalepsy induced by haloperidol, chlorpromazine, trifluoperazine and morphine. ASP and GLU produce a hyperthermic response in rats including the animal in which hypothermia was induced by reserpine.

REFERENCES

1. Aanonsen LM, Lei S, Wilcox GL. Excitatory amino acid receptors and nociceptive neurotransmission in rat spinal cord. *Pain* 1990; 41: 309–321.
2. Garlin AB, Sinor AD, Sinor JD, Jee SH, Grinspan JB, Robinson MB. Pharmacology of sodium dependent high affinity L–($_3$H) glutamate transport in glial cultures. *J Neurochem* 1995; 64: 2572–2580.
3. Salt TE, Hill RG. Neurotransmitter candidates of somatosensory primary afferent fibers. *Neurosciences* 1983; 10: 1083–1103.
4. Aanonsen LM, Wilcox GL. Nociceptive action of excitatory aminoacids in the mouse: Effects of spinally administered opioids, phencyclidine and sigma agonists. *J Pharmacol Exp Ther* 1987; 243: 9–19.
5. Malmberg AB, Yaksh TL. Spinal nitric oxide synthesis inhibition blocks NMDA-induced thermal hyperalgesia and produces antinociception in the formalin test in rats. *Pain* 1993; 54: 291–300.
6. Martinez DB, Kirchgessner AL, Butler PD, Bodner RJ. Monosodium glutamate and analgesia induced by morphine: Test specific effects. *Neuropharmacology* 1984; 23: 1141–1149.
7. Raigordosky G, Urca G. Intrathecal N-methyl-D-aspartate (NMDA) activates both nociceptive and antinociceptive systems. *Brain Res* 1987; 422: 158–162.
8. Zoccarato F, Valente M, Alexandre A. Hydrogen peroxide induces a long lasting inhibition of Ca^{2+} dependent glutamate release in cerebrocortical synaptosomes without interfering with cytosolic Ca^{2+}. *J Neurochem* 1995; 64: 2552–2558.
9. Davies OL, Raventos J, Walpole AL. Method for the evaluation of analgesic activity using rats. *Br J Pharmacol Chemother* 1946; 1: 255–264.
10. Jaffer WA, Griffith JQ. The central nervous system. Cisternal puncture. In "The rat in laboratory investigation". Ed Fareis, EJ: Griffith JQ. Hafner Publishing Company, New York, 1963, PP 196.
11. Balsara JJ, Jadhav JH, Muley MP, Chandorkar AG. Effect of drugs influencing central 5-Hydroxy–tryptaminergic machanisms on morphine induced catalepsy in rats. *J Pharma Pharmacol* 1979; 31: 255–257.
12. Miller KE, Clements JR, Larson AA, Beitz AJ. Organization of glutamate like immunoreactivity in the rat superficial dorsal horn. Light and electron microscopic observations. *Synapse* 1987; 2: 28–36.
13. Skilling SR, Smullin DH, Beitz AJ, Larson AA. Extracellular amino acid concentrations in the dorsal spinal cord of freely moving rats following veratridine and nociceptive stimulation. *J Neurochem* 1988; 51: 127–132.
14. Honey CR, Miljkovic Z, MacDonald JF. Ketamine and phensyclidine cause a voltage dependent block of responses of L-aspartic acid. *Neurosci Lett* 1985; 61: 135–139.
15. Marien M, Brien J, Jhamandas K. Regional release of ($_3$H)–dopamine from rat brain *in vitro* : effects of opioids on the release induced by potassium, nicotine and L-glutamic acid. *Can J Physiol Pharmacol* 1983; 61: 43–60.
16. Seeman P. Brain dopamine receptors. *Pharmacol Rev* 1980; 32: 229–313.
17. Loskota WJ, Schonbaum E. Reserpine : In "Body temperature regulation, drug effects and therapeutic implications" Ed Lomax P, Schonbaum E. Marcel Dekker, New York, 1979; PP 427–437.
18. Navarro CE, Cabrera RJ, Donoso AO. Interaction between glutamate and GABA on $_3$H- noradrenaline release from rat hypothalamus. *Brain Res Bull* 1995; 37: 119–122.
19. Baker CJ, Fiore AJ, Frazzini VI, Choudhri TF, Zubay GP, Solomon RA. Intraischemic hypothermia decreases the release of glutamate in the cores of permanent focal cerebal infarcts. *Neurosurgery* 1995; 36: 994–1001.