References

Arnt, J. (1985) Eur. J. Pharmacol. 113: 79–88
Braun, A. R., Chase, T. N. (1985) Soc. Neurosci. Abstr. 11: 671
Dolphin, A. C., Jenner, P., Sawaya, M. C. B., Marsden, C. D., Testa, B. (1977) J. Pharm. Pharmacol. 29: 727–734
Jackson, D. M., Hashizume, M. (1986) Psychopharmacology 90: 147–149
Jackson, D. M., Hashizume, M. (1987) J. Neural Transm. 69: 131–145
Mashurano, M., Waddington, J. L. (1986) Neuropharmacology 25: 947–949
Molloy, A. G., Waddington, J. L. (1984) Psychopharmacology 82: 409–410
Molloy, A. G., Waddington, J. L. (1985) Eur. J. Pharmacol. 108: 305–308
Robertson, G. S., Robertson, H. A. (1986) Brain Res. 384: 387–390
Yarbrough, G. G., McGuffin-Clineschmidt, J., Singh, D. K., Haubrich, D. R., Bendeski, R. J., Martin, G. E. (1984) Eur. J. Pharmacol. 99: 73–78

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High doses of L-naloxone but neither D-naloxone nor β-funaltrexamine prevent hyperthermia-induced seizures in rat pups

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Abstract—The effects of the non-specific opiate antagonist L-naloxone and the inactive isomer D-naloxone, as well as the specific mu receptor antagonist β-funaltrexamine, have been examined on hyperthermia-induced seizures in unrestrained 15 days old rats. Saline-injected animals exposed to an ambient temperature of 40°C showed a gradual increase in body temperature reaching a maximum of 42 ± 0·1°C at 50 min exposure. At this time all the pups had seizures and died. Similar results were obtained when the animals were pretreated with different doses of D-naloxone and β-funaltrexamine. Rats pretreated with L-naloxone also showed an increase in rectal temperature; but the temperature was lower than in saline-injected animals. Only high doses of L-naloxone prevented seizures and deaths. These data indicate that endogenous opioid peptides may play a role in seizures induced by hyperthermia and that receptors other than mu receptors could be involved in hyperthermia-induced seizures.

There is increasing evidence that endogenous opioid peptides may play a role in the pathophysiology of seizures, although pro- and anti-convulsant effects of opiates and endogenous opioid peptides have been reported (Frenk 1983; Tortella et al 1985). Both pro- and anti-convulsant effects could be explained by activation of different populations of opioid receptors. The existence of at least three distinct receptor types, mu, kappa and delta, has been suggested from in-vitro and in-vivo experiments, but that a particular response is mediated by a single subtype of opioid receptors has been difficult to establish, the major obstacle being the lack of selective opioid antagonists. Naloxone does not distinguish adequately between the different receptor types (Sawynok et al 1979). Recently, it has been suggested that the non-equilibrium antagonist β-funaltrexamine is selective for mu receptors (Ward et al 1982).

We have investigated the role of endogenous opioid peptides and opioid receptors, particularly the mu receptor (of which β-funaltrexamine is a selective antagonist) on seizures induced by hyperthermia in immature rats.

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Methods

Wistar albino rat pups aged 15 days and 34 ± 2·4 g were used. Acute hyperthermia was induced by placing rats in a ventilated incubator (UNITEMP) at a constant temperature of 40°C with 55% humidity. Rats were unrestrained during the experiments. Rectal temperatures were measured with a thermometer (ELLAB TF 3) connected to RM 6 thermocouple probes. The temperatures were recorded immediately after placing the rats in the chamber (time 0) and thereafter at 10 min intervals for a total of 90 min. The rats were injected intraperitoneally with 0·2 mL of 0·9% NaCl (saline), D-naloxone (1 or 10 mg kg^{-1}) and L-naloxone (1 or 10 mg kg^{-1}) at time 0; β-funaltrexamine was administered subcutaneously at a dose of 1·2 or 5 mg kg^{-1} 30 min before the experiments. The behavioural changes during exposure to hyperthermia were observed. The animals became hyperactive with scrambling movements in apparently random directions. After 30 min, generalized seizure activity appeared and this was followed by death.

Drugs used were: naloxone hydrochloride (Endo Laboratories Inc. New York, NY), β-funaltrexamine hydrochloride (Research Biochemical. Inc. Wayland, MA).

Data were analysed by Student's *t*-test and analysis of variance.

Results

Table 1 shows the results obtained with the different experimental groups. Saline-injected rats had a progressive increase in temperature reaching a maximal value of 42 ± 0·1°C at a 50 min exposure. Both groups injected with L-naloxone also showed a marked increase in body temperature, however, temperatures were significantly lower than in saline-injected animals ($P < 0·001$). The groups treated with D-naloxone and β-funaltrexamine did not show significant differences compared with the control group.

At 30 min, saline-injected animals had a temperature of 40·8 ± 0·2°C and 13% showed generalized tonic-clonic seizures (Table 1). In this group the rectal temperature was 42 ± 0·1°C after 50 min exposure and 100% of the animals had seizures.

The rats treated with L-naloxone 1 mg kg^{-1} (data not represented) had a rectal temperature of 40·9 ± 0·5°C at 50 min,

Table 1. Effects of L-naloxone (L-Nx), D-naloxone (D-Nx) and β-funaltrexamine (β-FNA) on temperature-induced seizures.

Time	Saline		L-Nx (10 mg kg^{-1})		D-Nx (10 mg kg^{-1})		β-FNA (5 mg kg^{-1})	
min	T°C	%S	T°C	%S	T°C	%S	T°C	%S
0	35·8±0·1	0	35·5±0·2	0	35·4±0·5	0	35·0±0·2	0
30	40·8±0·2	13	38·7±0·2	0	40·3±0·1	8	40·3±0·3	9
50	42·0±0·1	100	40·5±0·1	0	41·8±0·6	100	41·7±0·2	100
90	—	—	41·1±0·3	0	—	—	—	—

T°C = Rectal temperature
%S = Percent seizures
n = 15 rat pups for each group

and 100% suffered seizures. Animals receiving L-naloxone (10 mg kg^{-1}) reached a rectal temperature of 40·5±0·1°C after 50 min exposure to the 40°C environment, and no seizures or deaths were observed even after 90 min exposure (rectal temperature 41·1±0·3°C) in this group and all the rats had survived 24 h after the experiments.

At 50 min, the rectal temperature in the D-naloxone treated groups: (1 mg kg^{-1}, not shown, and 10 mg kg^{-1}) was 41·9±0·4°C and 41·8±0·6°C, respectively, and in both groups 100% of the animals had tonic-clonic seizures. The percentage of seizures in these groups were the same as in the control and L-naloxone (1 mg kg^{-1}) groups.

Animals injected with β-funaltrexamine 1·2 mg kg^{-1} (data not shown), or 5 mg kg^{-1}, demonstrated an increase in body temperature after 20 min exposure (39·7±0·2°C in both cases) which persisted throughout the observation period. At 50 min, the maximal temperatures were 41·6±0·13°C and 41·7±0·22°C, respectively. At this time, 100% of the animals showed generalized seizures (Table 1).

Discussion

Our results suggest that hyperthermia may produce changes in central nervous system transmitter release and/or binding to specific receptor sites that in the immature brain result in seizure activity. We believe that endogenous opioid peptides with proconvulsant activity are released during hyperthermia and produce seizures in the rat pups. The involvement of opioid receptors was indicated by the stereospecificity of naloxone's actions. Unlike L-naloxone, the inactive isomer D-naloxone (10 mg kg^{-1}) failed to prevent seizures in all cases. Our results confirm the hypothesis that endogenous opioid peptides may have a role in the development of seizures. However, due to the dose of L-naloxone needed to prevent hyperthermia-induced seizures and the results obtained with β-funaltrexamine (100% of rats showed seizures), it is possible that opioid receptors other than mu could be involved and consequently the endogenous opioid peptide released could be a delta or kappa agonist. Frenk (1983) suggested that endogenous opioid peptide-induced seizure activity is mediated by the delta-receptor subtype of cerebral opioid binding sites. However, Tortella et al (1984) reported that the mu receptor represents the primary binding site responsible for enkephalin-induced seizures. Moreover, Snead (1986) suggested that there may be no single "one receptor-one type of seizure" relationship in opiate-induced seizures.

Furthermore, hyperthermia may also differentially alter the affinity of opioid receptors for agonists and antagonists (Creese et al 1975). Therefore, we need to take into account the possibility that our high experimental temperature (40°C) could induce changes in mu receptor affinity. However, Puig et al (1987) demonstrated from in-vitro studies that the binding of naloxone to the mu receptor was not affected by changes in temperature.

However, it is not our purpose to suggest that endogenous opioid peptides are solely responsible for hyperthermia-induced seizures. The involvement of other neurotransmitter systems, in particular glycine and/or GABA (Werz & McDonald 1982), must be considered. Nonetheless, these experiments have provided comprehensive in-vivo data on the importance of endogenous opioid systems in the mechanism of seizures.

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References

Creese, I., Pasternak, G. W., Pert, C. B., Snyder, S. H. (1975) Life Sci. 16: 1837–1842
Frenk, H. (1983) Brain Res. Rev. 6: 197–210
Puig, M. M., Warner, W., Tang, C. K., Laorden, M. L., Turndorff, H. (1987) Br. J. Anaesth. In press
Sawynok, J., Pinsky, C., Labella, F. S. (1979) Life Sci. 25: 1621–1632
Snead, O. C. (1986) Exp. Neurol. 93: 348–358
Tortella, F. C., Robles, L., Mosberg, H. I., Holaday, J. W. (1984) Neuropeptides 5: 213–216
Tortella, F. C., Long, J. B., Holaday, J. W. (1985) Brain Res. 332: 174–178
Ward, S. J., Portoghese, P. S., Takemori, A. E. (1982) Eur. J. Pharmacol. 80: 377–384
Werz, M. A., McDonald, R. L. (1982) Brain Res. 236: 107–119