SELECTIVE BLOCKADE OF mGlu5 RECEPTORS ON DORSAL RAPHE 5-HT RELEASE IN MODELS OF TONIC, INFLAMMATORY AND CRONIC PAIN

Genovese R., Dottorato di Ricerca in Scienze Farmacologiche e Fisiopatologia Respiratoria, XVI ciclo, Durata del Dottorato in anni: 4. Sede di servizio: Department of Experimental Medicine, Section of Pharmacology "L. Donatelli", Second University of Naples, Napoli.

Introduction

Dorsal raphe (DR) serotonin (5-HT) neurons are involved in antinociceptive function by facilitation of pain inhibitory descending system. In addition to 5-HT, glutamate evokes antinociception from several brain structures including DR. Among glutamate receptors involved in controlling nociception metabotrobic glutamate subtype 5 ($mGlu_5$) has recently been indicated as pharmacological target to relieve pain, since its peripheral, spinal and cerebral selective blockade produces antinociception. Thus, analgesic potential of the selective antagonist of these receptors, 2-methyl-6-(phenylethynyl)-pyridine (MPEP), which penetrates the blood-brain barrier when systemically administrated, (Gasparini et al 1999) was investigated evaluating the DR 5-HT release and correlating these changes to nociceptive
behaviour. *In vivo* microdialysis has been carried out simultaneity to plantar test in three models of pain in rat.

Methods

The formalin and carrageenan tests have been used as models of persistent pain and inflammatory pain respectively. The partial sciatic nerve ligation model of neuropathic pain was used to produce chronic hyperalgesia in rats.
Male Wistar rats were anesthetized and implanted with concentric dialysis probes in the dorsal raphe. The day after surgery, each implanted probe was perfused with artificial cerebrospinal fluid at a rate of 0.8 µl/min using an infusion pump. After an initial 60 min equilibration period, dialysate samples were collected every 30 min. Formalin or carrageenan were injected into the hind paw after the collection of the fifth post-equilibration dialysate. The sciatic nerve was exposed at mid-thigh and freed of connective tissue, four ligatures of 0.4 gauge chromic gut were tied loosely around the nerve. MPEP was administered by intraperitoneal route (i.p.) at the dosage of 5 mg/ml/Kg alone or 30 min before injection of formalin or carrageenan or after the fifth perfusate sample in the neuropathic rat. 5-HT was determined using HPLC. The nociceptive behaviour was also monitored by plantar test. The latency of nociceptive reaction was measured in seconds each 30 min under basal condition and after drug treatment and/or inducing pain. Results of the plantar test have been expressed as percentage of the maximum possible effect (% MPE). Sections (50 µm) of DR were incubated with primary antibodies to rabbit anti-protein gene product (PGP) 9.5 to mark specific neurons activity (Wilkinson et al. 1989).

Results

Subcutaneus injection of formalin (5% 50 µL) determined a significant ($P<0.05$) increase of 5-HT release (137±4% *vs* basal value) and a significant reduction of nociceptive response (NR) of -40±7%, expressed as % of the maximum possible effect (MPE). Pretreatment with MPEP (5 mg/kg i.p.) led to a further and significant increase of 5-HT (180±12% *vs* basal value), but did not reduce the NR (-15±7% MPE). Intraplantar

injection of carrageenan significantly increased extracellular 5-HT (212±16% *vs* basal value) and led to significant thermal hyperalgesia (-65±3% MPE). Even in this case, pretreatment with MPEP significantly enhanced carrageenan-induced 5-HT release (260±18% *vs* basal value) and decreased carrageenan-induced hyperalgesia (-36±9% MPE). Rats with partial ligature of the sciatic nerve (CCI) showed thermal hyperalgesia. CCI rats had higher level of extracellular 5-HT (300±23 % *vs* basal value) than the sham-operated (135±17% *vs* basal value). Moreover, higher DR PGP 9.5 immunoreactivity (159±13) compared to sham operated animal (96±11) was also observed. Pretreatment with MPEP reduced extracellular 5-HT both in CCI rats and in the sham up to 86±12 % and 57±17% *vs* basal value, respectively. MPEP, per se, reduced significantly extracellular 5-HT (44±11% *vs* basal value) and increase the latency of NR (44±6 % MPE).

Conclusion

This study shows that $mGlu_5$ receptors modulate the activity of DR serotonergic neurons and provides evidence that $mGlu_5$ receptors blockade may generate specific changes in DR 5-HT extracellular values in models of inflammatory and neuropathic pain in rat. In inflammatory pain models, selective blockade of these receptors led to analgesia probably by a 5-HT-mediated facilitation of the endogenous antinociceptive pathway, while in neuropathic pain conditions MPEP is unable to relieve pain and decreases 5-HT release.

References

1. Gasparini F, Lingenhöhl K, Stoehr N et al. Neropharmacology 1999a; 38: 1493-1503.

2. Wilkinson KD, Lee KM, Deshpande S, Duerksen-Hughes P, Boss JM, Pohl J. Science 1989. 246: 670-673.