Life Sciences, Vol. 60, No. 16, pp. 1359-1363, 1997

0024-3205/97 $17.00 + .00

ELSEVIER

PII S0024-3205(97)00081-7

THE EFFECTS OF AN INTRATHECAL NMDA ANTAGONIST(AP5) ON THE BEHAVIORAL CHANGES INDUCED BY COLORECTAL INFLAMMATION WITH TURPENTINE IN RATS

Yasuo Ide, Yasuhiro Maehara, Sumio Tsukahara, Luke M. Kitahata and J.G. Collins

Department of Anesthesiology, Yale University School of Medicine, New Haven, CT 06510

(Received in final form January 17, 1997)

Summary

Visceral pain, especially that associated with inflammation of visceral organs, is poorly understood and difficult to treat clinically. The purpose of this study was to investigate the effects of intrathecal 2-amino-5-phosphonovaleric acid (AP5, a competitive NMDA antagonist) upon a visceromotor response to distension of colonic tissue inflamed by exposure to turpentine. All experiments were conducted under pentobarbital anesthesia. Animals were prepared with a laminectomy from T12 to L1 to facilitate intrathecal drug administration. Colonic distension thresholds for a visceromotor response were determined in the presence and absence of AP5. Animals were divided into two groups. The NS group received 50 μl of saline intrathecally and the AP5 group 10 mM of AP5 in 50 μl saline. After baseline measurements, intrathecal drugs were administered. Five minutes later, the effects of intrathecal drugs were measured, then 1 ml of 25% turpentine was administered anorectally. Subsequent measurements were made every 5 minutes for the next 90 minutes. Visceromotor thresholds to colorectal distension (CRD) were significantly decreased 50 min after turpentine administration in the NS group. There was no threshold change in the AP5 group. This study suggests that the administration of the competitive NMDA receptor antagonist AP5 in this model blocks the effect of turpentine sensitization on visceromotor response to CRD. The absence of AP5 effects in animals not sensitized by turpentine suggests that NMDA systems may be involved in the sensitization.

Key Words: 2-amino-5-phosphonovaleric acid, colorectal distension, nociception, sensitization, dorsal horn neuron

As efforts to understand how the nervous system responds to stimuli that are capable of producing pain become more sophisticated, we are forced to more closely consider differences between experimental and clinical pain. An important component of many clinical pain states is inflammation. It is clear that a painful stimulus may sensitize elements of the nervous system so that subsequent responses are heightened (hyperalgesia or allodynia) and that inflammation may be a mechanism of such sensitization (1-11). A large body of literature exists that deals with somatic pain. More recently, focus has begun to be placed on visceral pain (12-13). Not only such

work of academic interest, but there are clinical situations, such as irritable bowel syndrome, that may result from inflammation induced sensitization of the viscera (14-18). The NMDA receptor has been shown to play an important role in modulating pain, especially at the level of the spinal cord. This study was designed to examine the effects of a competitive NMDA antagonist [2-amino-5-phosphonovaleric acid(AP5)] on the response to CRD of colons sensitized by local application of turpentine.

Methods

This protocol was approved by the Yale Animal Care and Use Committee, and institutional, state and federal guidelines for humane care and use of laboratory animals were observed during all aspects of this study. The experiments were performed in 32 adult male Sprague-Dawley rats weighing 320-540 g. Animals were initially anesthetized with intraperitoneal pentobarbital (Nembutal®) 40 $mg \cdot kg^{-1}$. If within 10 min animals responded to pinching the skin on the abdomen, an additional 20 $mg \cdot kg^{-1}$ Pentobarbital was administered intraperitoneally. The average doses of intraperitoneal pentobarbital was 46.7 mg. Following tracheostomy, an external jugular vein and an internal carotid artery were cannulated for fluid and drug administration and for monitoring of arterial blood pressure, respectively. Approximately 30 minutes after intraperitoneal injection, an administration of intravenous pentobarbital was started at a mean rate of 8.7 $mg \cdot kg^{-1} \cdot hr^{-1}$. This amount of pentobarbital was sufficient to maintain adequate level of anesthesia for surgical preparation. The laminectomy from T_{12} to L_1 was performed and the vertebral column was mounted on a rigid frame. The dura mater was carefully cut using stereoscopic microscope and the spinal cord was covered with warm mineral oil. Laminectomy enabled us to administer the test drug intrathecally and also provided baseline data for comparison with planned electrophysiological studies. During a one hour period after completion of laminectomy, a proper level of anesthesia was established before the study was begun. In order to maintain proper level of anesthesia throughout the experiments described by Sinclair et al. (19) or Carstens et al. (20,21), namely the absence of spontaneous movement, corneal, auricular and pinnal reflexes, except for the presence of limb flexion reflex, the dose of intravenous pentobarbital was increased by a mean rate of 1.7 $mg \cdot kg^{-1} \cdot hr^{-1}$ if spontaneous movement was present or decreased by a mean rate of 1.7 $mg \cdot kg^{-1} \cdot hr^{-1}$ if the reflex was absent. The average dose of pentobarbital to keep this level of anesthesia was 12.5 $mg \cdot kg^{-1} \cdot hr^{-1}$ (8.3 -17.5 $mg \cdot kg^{-1} \cdot hr^{-1}$). Body temperature was monitored with an esophageal probe and maintained within normal limits. Physiological parameters of the animals were maintained within normal limits.

Stimulus parameter: CRD was used as a noxious visceral stimulus. The method of CRD we used is similar to that described by Ness and Gebhart (22). Distention of the descending colon and rectum was achieved by a pressure controlled air inflation of a 6 cm long distention balloon inserted intra-anally (22 - 25). The distention balloon was connected to a pressure controlled balloon inflator (26) through a balloon catheter and was inflated at a rate of 4 mmHg / sec beginning at 0 mmHg until an 80 mmHg maximum in 20 seconds was reached. A small detection balloon (1-1.5 cm-long, flexible, latex) was attached distal to the tip of the distention balloon catheter to monitor changes in intraluminal pressure (27). It was filled with 0.7 ml of air to monitor intraluminal pressure which was found to be stable at this level of anesthesia. In order to minimize the effects of manipulation, the device used in the sensitization study had additional lumen for the administration of turpentine. Turpentine was used for sensitization of the colorectum (12,13). Turpentine with peanut oil(1 ml of 25% solution) was injected into the colon and rectum through the catheter, 5 minutes after the intrathecal administration of AP5 or saline. This allowed for a post intrathecal administration value to be obtained prior to sensitization. Animals were divided into two groups, one was treated intrathecal AP5 injection (n=17), the other was treated intrathecal

normal saline injection (n=17). AP5 was dissolved with normal saline to make 10 mM solution, which was reported as the highest dose without muscle dysfunction (28). Abdominal muscle contraction in response to CRD, which, visceromotor response (VMR) was signaled by the detection balloon with a sudden rise in pressure. The pressures within the detection and distention balloon were recorded on a chart recorder simultaneously. The intraluminal pressure(3-5 mmHg) was set at zero scale on the recording chart. The criteria for identifying a response is as follows. A sudden rise in the intraluminal pressure followed by continual rise was detected on the recording chart. At the same time, muscular contraction was observed visually which verified the rise in the intraluminal pressure seen on the recording chart. The baseline contraction of abdominal and hindlimb musculature in response to three CRD separated by 5 minute interstimulus intervals were measured and averaged. After the baseline observation, 50 μl of normal saline (NS group) or AP5 (AP5 group), was administered intrathecally(29). The CSF and mineral oil on the surface of the cord was removed and drug or saline was administered with a microsyringe under stereoscopic microscopic observation. 5 minutes after the administration, pre-sensitization value was determined. Then, turpentine was administered intraluminally for the sensitization. The CRD was done every 5 minutes thereafter up to 90 minutes after turpentine administration. The threshold stimulation utilized in the present study evoked a steady and constant response to the CRD. Suprathreshold stimuli which might have caused ischemia of the intestinal wall and might have compromised the steady state response was not used in this study.

Repeated measures ANOVA was used to compare the time effect of each group. T-test for independent samples was used to compare control and pre-sensitization values between NS group and AP5 group. With an indication of significance by ANOVA, additional analysis was done. The control value and pre-sensitization value were compared to 90 minutes after sensitization study value using a paired t-test in each group. T-test for independent samples was used to compare the mean difference between control and the 90 minutes after sensitization data and between pre-sensitization and 90 minutes after sensitization data. Differences were determined to be significant with p values less than 0.05. All data were presented as mean±SE.

Results

Fig.1 shows the time course of the VMR threshold for CRD of NS and AP5 group. The control VMR threshold for CRD of the control group and AP5 group were 21±1.1 mmHg and 20±1.2 mmHg, respectively. There was no difference in mean control values between the NS group and AP5 group. Likewise there was no difference at 5 minutes after AP5 or NS administrated time point.

Effect of sensitization on behavioral response to CRD: After the administration of turpentine in the NS group, the VMR threshold were gradually decreased. The threshold dropped to about 10 mmHg at 60 min after the administration of turpentine, and was stable until the end of study (90 min after the administration of turpentine). There was a significant time effect (F with 19, 326 DF=7.60, p=0.0001). There was a significant difference between the mean value at 90 minutes after sensitization and the mean control value (p=0.0001) and the mean pre-sensitization value (p=0.0001).

Effect of intrathecal AP5 on sensitization of behavioral response to CRD: The thresholds for CRD of AP5 group were not changed following the administration of turpentine. There was no significant time effect (F with 19, 287 DF=0.70, p=0.82) and there was no significant difference between the mean value at 90 minutes after sensitization and the mean control value and that of pre-sensitization, respectively.

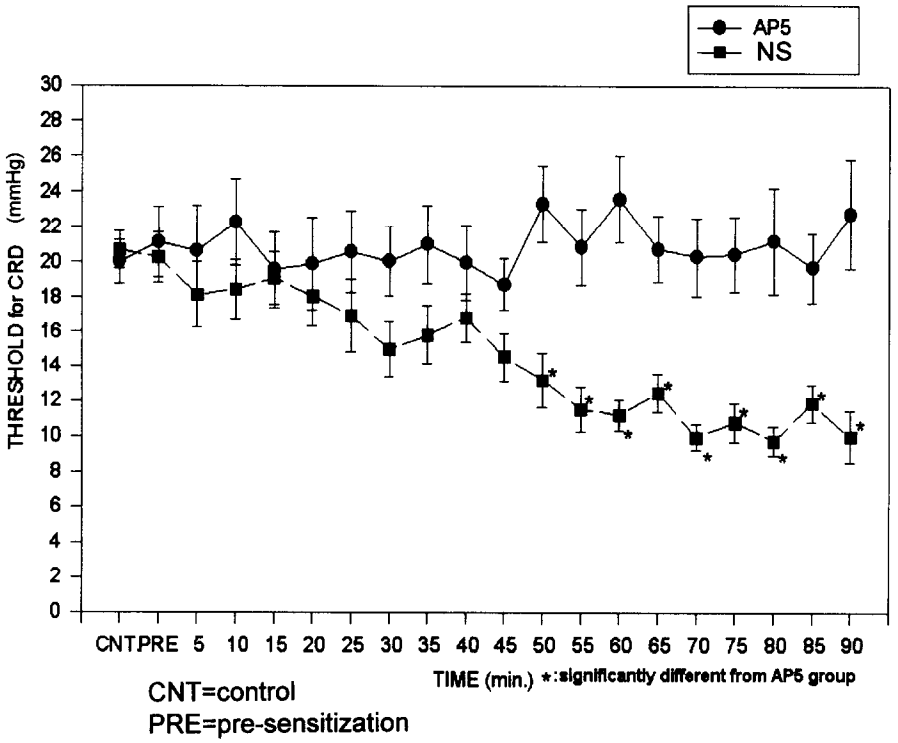

Fig 1.

The mean difference between control value and 90 minutes after sensitization was significantly larger for the NS group than for the AP5 group. The mean difference between pre-sensitization and 90 minutes after sensitization was significantly larger for the NS group than for the AP5 group. The group means started to become different at 50 minutes after sensitization study and remained significantly so through 90 minutes after sensitization study ($p<0.0025$ for 50 minutes study through 90 minutes study).

Discussion

In this study, the colorectal administration of the turpentine induced a significantly decreased VMR threshold for CRD. Intrathecally administered AP5 appeared to block that decrease in threshold. McMahon and Abel (7) reported that 25% turpentine applied to bladder induced inflammation, hyperreflexia, decreased micturition threshold and the hypersensitivity at tail and lower abdomen. Rice and McMahon (10) reported that this visceral pain induced by turpentine was prevented by high dose (> 250 $\mu g \cdot 50\mu l^{-1}$(25 mM)) preemptive intrathecal administration of AP5. Our results showed 10 mM(99 $\mu g \cdot 50\mu l^{-1}$) was enough to prevent sensitization of CRD, a concentration compatible with that used in somatic studies (28).These results suggest that turpentine sensitization of viscera may involve NMDA mechanisms. They also suggest that analgesics that act through NMDA receptor may be appropriate agent as visceral pain medication.

Acknowledgment

The authors acknowledge assistance by Theresa O'Connor Ph.D. for the statistical data. This study was supported by NIH Grant NS-09871 to L.M.K

References

1. M. MIAMPAMBA, S. CHÉRY-CROZE, F. GONY, F. BERGER, J-A. CHAYVIALLE, Pain 57 327-334 (1994).
2. H-G. SCHAIBLE, R. F. SCHMIDT, J. Neurophysiol. 54 1109-1122 (1985).
3. H-G. SCHAIBLE, R. F. SCHMIDT, W. D. WILLIS, Exp. Brain Res. 66 489-499 (1987).
4. H-G. SCHAIBLE, R. F. SCHMIDT, J. Neurophysiol. 60 2180-2195 (1988).
5. J. L. K. HYLDEN, R. L. NAHIN, R. J. TRAUB, R. DUBNER, Pain 37 229-243 (1989).
6. V. NEUGEBAUER, T. LUECKE, H-G. SCHAIBLE, J. Neurophysiol. 70 1365-1377 (1993).
7. S. B. MCMAHON, A. ABEL, Pain 28 109-127 (1987).
8. S. B. MCMAHON, Agents Actions 25 231-233 (1988).
9. H. J. HAEBLER, W. JAENIG, M. KOLTZENBURG, J. Physiol. (London) 425 545-562 (1990).
10. A. S. C. RICE, S. B. MCMAHON, Pain 57 335-340 (1994).
11. D. W. GAMNISON, M. J. CHANDLER, R. D. FOREMAN, Pain 49 373-382 (1992).
12. T. J. NESS, G. F. GEBHART, Pain 41 167-234 (1990).
13. T. J. NESS, A. RANDICH, G. F. GEBHART, Neurosci. Letters 131 113-116 (1991).
14. J. RITCHIE, Gut 14 125-132 (1973).
15. A. PRIOR, D. G. MAXTONJ, P. J. WHORWELL, Gut 31 458-462 (1990).
16. W. E. WHITEHEAD, B. HOLTKOTTER, P. ENCK, R. HOELZL, K. D. HOLMES, Gasteroenterology 98 1187-1192 (1990).
17. E. A. MAYER, H. E. RAYBOULD, Gasteroenterology 99 1638-17024 (1990).
18. E. A. MAYER, G. F. GEBHART, Gasteroenterology 107 271-293 (1994).
19. J. G. SINCLAIR, C. D. MAIN, G. F. LO, Pain 33 357-362 (1988).
20. E. CARSTENS, M. HARTUNG, B. STELZER, M. ZIMMERMANN, Pain 43 105-112 (1990).
21. E. CARSTENS, I. G. CAMPELL, Pain 48 215-226 (1992).
22. T. J. NESS, G. F. GEBHART, Brain Res. 450 153-169 (1988).
23. T. J. NESS, G. F. GEBHART, J. Neurophysiol. 60 419-1438 (1988).
24. T. J. NESS, G. F. GEBHART, J. Neurophysiol. 62 220-230 (1989).
25. T. J. NESS, G. F. GEBHART, Brain Res. 486 301-309 (1989).
26. R. H. AND,ERSON, T. J. NESS, G. F. GEBHART, Physiol. Behav. 41 635-638 (1987).
27. Y. HARADA, K. NISHIOKA, L. M. KITAHATA, J. G. COLLINS, Anesthesiology 83 336-343 (1995).
28. G. RAIGORODSKY, G. URCA, Brain Res. 422 158-162 (1987).
29. A. H. DICKENSON, A. F. SULLIVAN, Brain Res 506 31-39 (1990).