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Possible Involvement of Endogenous Opioid Peptides in the Mechanism of Dantrolene Actions

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ABSTRACT. The effects of dantrolene on the release of opioid substances were studied in three different biological systems. It was observed that dantrolene possesses an antinociceptive activity, and an inhibitory action on the contractions of the rabbit's jejunum, and frog's rectus abdominis muscles. All of these actions were antagonised by naloxone in a noncompetitive manner. These observations may indicate that endogenous opioids are involved in the mechanism of dantrolene actions.

KEY WORDS: Dantrolene, endogenous opioids, antinociception

Calcium plays an integral role in the release of several neurotransmitters[1-3]. Further, endogenous opioid peptides were suggested to be involved in the regulation of the release of some neurotransmitters[4-8]. Therefore, compounds which interfere with calcium movement may modulate the release of neurotransmitters and, hence, may effect the release of endogenous opioid peptides.

Dantrolene is a drug which interferes with intracellular calcium transport[9,10]. Thereby, it may modulate the release of some neurotransmitters. Hence, dantrolene may affect the mechanism which regulates the release of these transmitters, in which opioid peptides were suggested to be involved[4-8]. Therefore, it is possible that dantrolene interferes with the release of endogenous opioid peptides. This is further indicated by the reported interaction of dantrolene and opioid peptides in heat stroke attacks[11-14].

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In view of the above, the interaction of dantrolene and the opioid system was investigated by studying the effect of dantrolene on nociception in mice. The interactions of dantrolene and naloxone on isolated smooth and skeletal muscles were also studied.

Material and Methods

1. Antinociception Testing

Antinociception was tested using the mouse hot plate test in essentially the same manner as described by Woolfe and Macdonald[15]. In this test, the mouse was put on a hot surface (stainless steel plate floating on a water bath set at 55.5°C). It is assumed that the mouse jumps or licks its extremities when it feels pain. The reaction time was defined as the period from placing the mouse on the hot plate until it feels pain.

Male swiss mice weighing 20-25 gm were used in this investigation. All mice were screened for their reaction time, only mice which showed a reaction time of 10 seconds or less were used in this experiment. Animals were used in groups of 20 mice, each group received dantrolene sodium (7.4-66.9 μMol/kg, Dantrium^R, Norwich Eaton), and/or naloxone hydrochloride (13.7 or 27.4 μMol/kg, Narcan^R, Dupont Pharmaceuticals). Control groups were given the vehicle present in the pharmaceutical formulation of dantrolene which was used in this study (alkaline mannitol solution at the same concentration present in Dantrium^R). All drugs were injected intraperitoneally just before the beginning of the experiment. The volume of injection given to each animal was always less than 0.5 ml. Antinociception was tested at one hour after injection. A reaction time of 30 seconds or more was considered as the criterion for antinociception. In each group, the percentage of animals showing antinociception was calculated. The results of all groups were tested for significance using the normal approximation to the binomial distribution.

2. Isolated Rabbit Jejunum Experiments

White male Newzealand rabbits weighing 3-4 kg were used in this experiment in the same manner as described by Mahmoudian *et al.*[16]. A piece of the upper part of the jejunum was isolated and bathed in 37°C oxygenated Tyrode solution in a 5 ml bath. After 30 min of equilibration, appropriate cumulative amounts of dantrolene solution in polyethylene glycol (38-228 μM) were added to the bath. Naloxone hydrochloride solution (4.4 or 11 μM) was added in some experiments 3 min before dantrolene addition. Control experiments were performed with dantrolene vehicle in the same manner as described above. Peristaltic contractions were recorded by a Palmer Bioscience potentiometric recorder connected to the muscle through a Bioscience UFI isometric transducer and A100 Bioscience coupler. For each treatment group, the experiment was repeated six times. Percentage inhibition of contraction in each experiment was calculated in reference to the control contraction before drug addition. Results of all groups were averaged. Averages of different treatment regimens were compared to each other by the student's test.

3. Frog Rectus Abdominis Muscle Experiments

Frogs weighing 25-50 gm were used in these experiments. The rectus abdominis

muscle of the frog was isolated and suspended in a 5 ml organ bath. The preparation was left to equilibrate for 30 min at room temperature. Acetylcholine (ACh, 5 μM) was used as an agonist to elicit muscle contractions. It was left in contact with the muscle for 90 seconds. For each muscle, a dose response curve for ACh was recorded. Then, a dose of ACh that causes a submaximal contraction, was selected for the rest of the experiment. Dantrolene (38-228 μM) or its vehicle were added 1 min before the addition of the selected ACh dose. Naloxone (4.4 or 11 μM) was added 3 min before the addition of other drugs. Muscle contractions were recorded, calculated, and analyzed in the same manner as described above in the rabbit experiments.

Results

1. *Antinociceptive Activity of Dantrolene*

Figure 1 shows the effect of dantrolene on nociception. It was observed that dantrolene, in a dose-related manner, had an antinociceptive activity in the mouse hot plate test. This activity reached a maximum when 80% of animals showed antinociception. On the other hand, groups which received mannitol (vehicle present in Dantrium^{R}) did not show any antinociception.

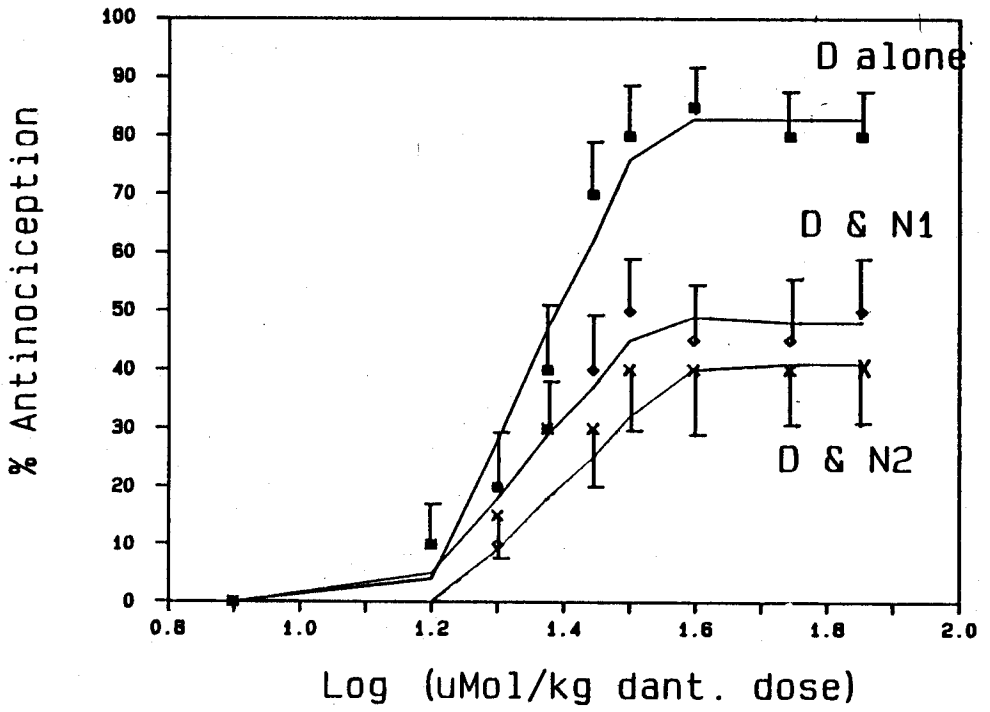

FIG. 1. Log dose response curve of the antinociceptive effect of dantrolene alone (D), and in the presence of 13.7 μMol/kg naloxone (D & N1), or 27.4 μMol/kg naloxone (D & N2). Each point represents the percentage of a group of 20 mice showing an action. Lines above or below points represent a one side 95% confidence interval.

The antinociceptive activity of dantrolene was decreased significantly in the presence of naloxone (5 and 10 mg/kg). The maximal percentage of animals showing antinociception due to dantrolene in the presence of these doses of naloxone was 40-50%. This is significantly less than the observed maximal activity of dantrolene in the absence of naloxone ($p < 0.01$). Although the response to the higher dose of naloxone seems to be more than the response to the lower dose, this observation was not statistically significant ($P > 0.05$).

2. *Isolated Rabbit Jejunum Experiments*

Figure 2 shows the percentage inhibition of contractions of the rabbit jejunum in response to various doses of dantrolene in the absence or the presence of naloxone (4.4 or 11 μMol/L). It is observed that dantrolene caused a dose related inhibition of these contractions. This observed inhibition was prevented by previous addition of naloxone. The maximal percentage of inhibition of contraction in response to dantrolene alone was 70-80%, while in the presence of naloxone, the maximal percentage of inhibition was 55-65%. This is significantly less than the observed maximal inhibition in the absence of naloxone ($P < 0.01$). Although the response to the higher dose of naloxone seems to be more than the response to the lower dose, this was not statistically significant. Naloxone alone, in the used concentrations, did not show any actions in this experiment.

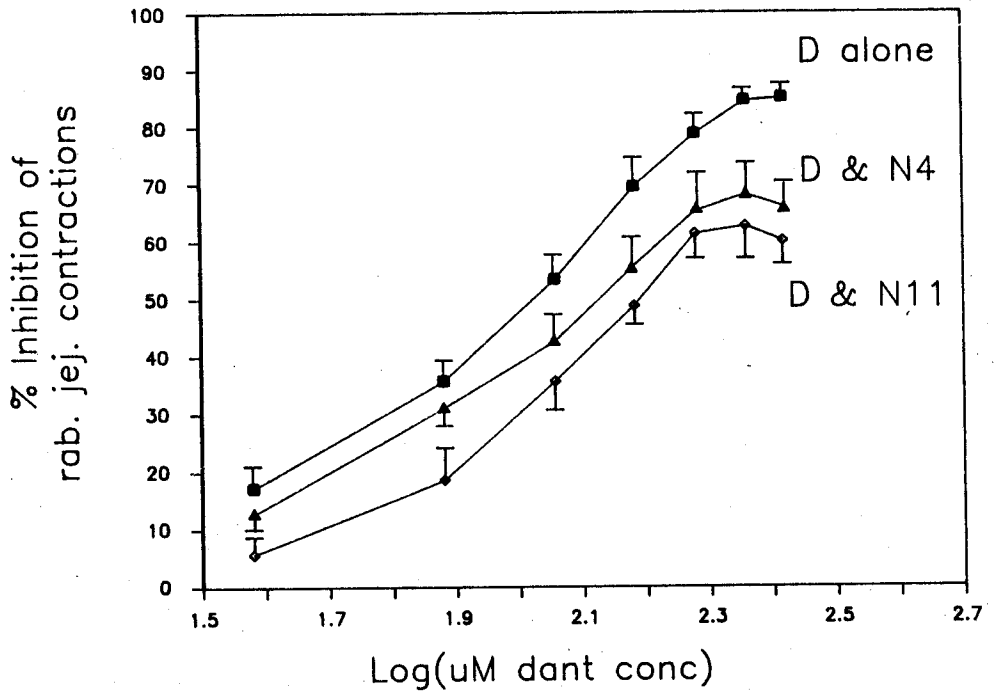

FIG. 2. Log dose response curve of the inhibition of rabbit jejunum contraction by dantrolene alone (D), and in the presence of 4.4 μM naloxone (D & N4), or 11 μM naloxone (D & N11). Each point is the mean of 6 experiments. Lines above or below points represent a one side standard deviation.

3. Frog Rectus Abdominus Muscle Experiments

Figure 3 shows the percentage inhibition of ACh-induced contractions of the frog rectus abdominis muscle. It is clear that dantrolene inhibited these contractions in a dose-related fashion. This observed inhibition of contractions was decreased significantly in the presence of naloxone ($P < 0.01$). The maximal percentage of inhibition of contractions induced by dantrolene alone was 75-85%, while in presence of 4.4 μMol/L naloxone it was 45-55%. It was further decreased significantly to 25-35% in the presence of 11 μMol/L naloxone ($P < 0.01$). Neither mannitol nor naloxone when tested alone, in the used concentrations, showed any action in this experiment.

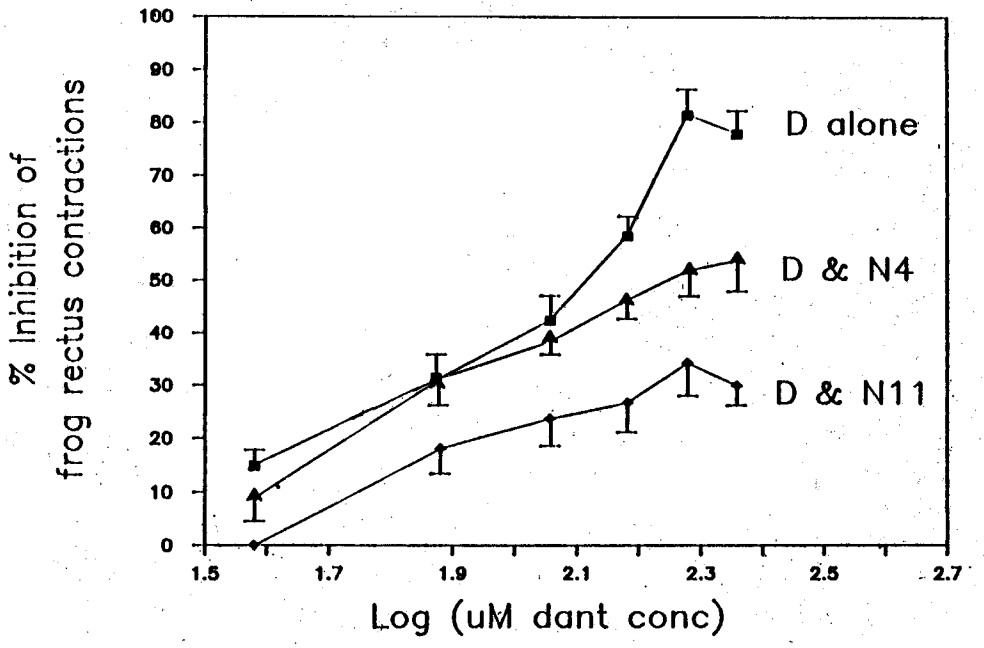

FIG. 3. Log dose response curve of the inhibition of frog rectus abdominis muscle contraction by dantrolene alone (D), and in the presence of 4.4 μM naloxone (D, & N4), or 11 μM naloxone (D & N11). Each point is the mean of 6 experiments. Lines above or below points represent a one side standard deviation.

Discussion

In this investigation, dantrolene inhibited, in a dose-related manner, the contractions of smooth and skeletal muscles. It also inhibited nociception in a comparable manner. Furthermore, naloxone prevented these actions of dantrolene and caused a downward shift of their dose response relationships. From the shape of the curves, it is thought that this antagonistic effect of naloxone is non-competitive.

The observed actions of dantrolene on nociception and on the contraction of skeletal and smooth muscles are similar to the reported actions of opiates[17]. Further, both morphine and dantrolene have similar effects in decreasing calcium release[9,10,17]. Therefore, it is thought that dantrolene actions are probably mediated through its interaction with the endogenous opioid system. This is further indicated by the observed antagonistic effect of naloxone to dantrolene actions.

There exist two possibilities for the nature of dantrolene interaction with the opioid system. The first possibility is *via* a direct binding of dantrolene to the opiate receptors. This possibility may be a little remote, because naloxone antagonized dantrolene activity in a non-competitive fashion, while it is known that naloxone is a competitive antagonist of the opiate receptors. The other possibility is *via* inducing the release of an endogenous opioid substance(s). This would imply the presence of two separate sites of actions for dantrolene and naloxone, which may explain the non-competitive nature of dantrolene-naloxone interaction.

It is possible that dantrolene causes the release of endogenous opiates as the first step in its action. Then the released endogenous opiates cause the decrease in the release of calcium, which is considered as the main action of dantrolene. However, it is also possible, that dantrolene *via* its actions on calcium movement, causes a disturbance in the release mechanism of some neurotransmitters, leading to an imbalance of the level of these neurotransmitters which in turn would release endogenous opiates.

More studies are required to elucidate the precise involvement of endogenous opioid peptides in the mechanism of action of dantrolene.

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احتمال وجود دور لببتيدات المورفين الداخلة في آلية تأثير الدَّنترولين

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المستخلص . تمت دراسة تأثير الدَّنترولين على إفراز المواد الشبيهة بالمورفين في ثلاث منظومات حيوية . وقد لاحظنا أن الدَّنترولين له تأثيرات مضادة للشعور بالألم ، وتأثيرات مثبطة لانقباضات عضلات الأرانب المعوية وعضلات الضفدعة البطنية . كل هذه التأثيرات للدَّنترولين أمكن منعها بالنالوكسون بطريقة غير تنافسية . هذه المشاهدات والنتائج قد تشير إلى وجود دور لمشتقات المورفين الداخلة في آلية تأثير الدَّنترولين .