Effects of D-α-aminoadipate on physiologically evoked responses of cat dorsal horn neurones

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Summary. Microelectrophoretic administration of D-α-aminoadipate reversibly reduced excitatory responses of cat dorsal horn neurones evoked by iontophoretic glutamate and non-noxious peripheral stimuli but did not influence similar responses evoked by noxious peripheral stimuli or iontophoretic substance P.

The recent suggestion that D-α-aminoadipate (DαAA) may be an antagonist at excitant amino-acid receptor sites in the CNS[2] has been confirmed in several preliminary studies[3,4]. This compound has little or no effect on responses to carbachol, substance P or noradrenaline in vitro[3,4] but in vivo depresses excitation by l-aspartate somewhat more readily than l-glutamate[3,4] and discriminates even more clearly between the actions of kainate and N-methyl-D-aspartate, which may act preferentially on 'glutamate-prefering' and 'aspartate prefering' receptors[5,6].

We have utilized D-α-aminoadipate in microelectrophoretic experiments in the cat dorsal horn in order to investigate the possibility that neurones in this region, activated by physiological stimulation of the periphery, use an excitatory amino-acid neurotransmitter.

Methods. Experiments were performed in adult cats anaesthetized with pentobarbitone (35 mg/kg i.p. initially, supplemented i.v. when necessary) and acutely spinalized at L1. Dorsal horn neurones were located in segments L6-S1, following their activation by noxious (radiant heat) or non-noxious (air jets) stimulation of the ipsilateral hind-foot (see Davies and Dray[7] for details). The anatomical location of cells was determined by dye marks (pontamine sky blue or acid fast green) found in frozen sections (80 µm) of spinal cord, cut within 24 h of completing the experiment.

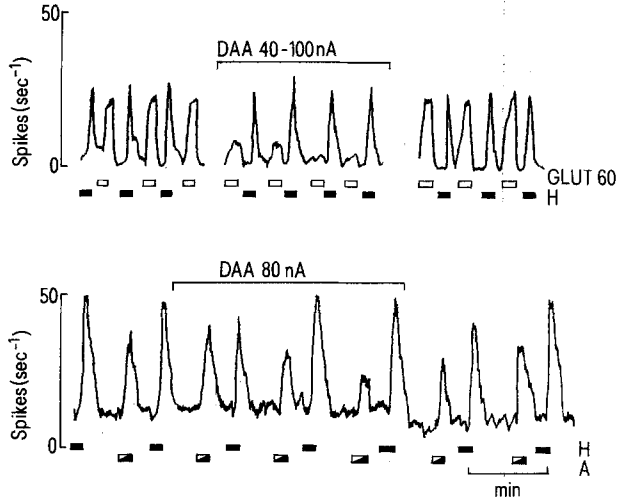
Conventional methods were used to eject drugs by electrophoresis. Extra-cellular recordings of single neurone activity were made through the 4 M NaCl-filled centre barrel of a multibarrelled micropipette; other barrels contained the following substances: sodium L-glutamate 0.5 M, pH 7.0; sodium D-α-aminoadipate 0.2 M, pH 7.0, substance P (SP 0.0007 M in 0.165 M NaCl) pontamine sky blue (2% in 0.5 M Na acetate) or acid fast green (saturated solution in 0.165 M NaCl). The influence of DαAA on the submaximal responses of dorsal horn neurones activated by both noxious and non-noxious peripheral stimulation or by electrophoretically administered l-glutamate or SP was studied.

Results. DαAA ejected at 40–100 nA (mean 60 nA) for 3–12 min (mean 5 min) produced a reversible reduction, by 40–80% (mean 62%) of the responses evoked by non-noxious stimuli on 8 of 9 dorsal horn neurones studied (2 cells lamina I; 4 cells lamina IV and 3 cells lamina V). The chemical excitation of these cells by electrophoretic l-glutamate (40–80 nA, 5 cells) was also reduced, but the responses to noxious stimuli (7 cells) were unaffected (figure 1). In only 1 cell was there a modest (by 20%) reduction of the response to noxious stimuli. The latency to onset of the effect of DαAA in these experiments varied between 1 and 3 min, but recovery in all cases was rapid, usually occurring within 1 min of terminating the ejection. In all these studies DαAA did not modify neuronal spike configuration but did produce a slight reduction of background firing (by 15%) in 3 cells. SP (40 and 60 nA) only excited 2 cells reproducibly and this excitation was unaffected by DαAA.

In further experiments DαAA was ejected into lamina II–III (substantia gelatinosa), from a micropipette which had been successfully used in the experiments described above, while recordings were being made via an independent micropipette positioned some 100 µm away in lamina IV or V. In these experiments substantially greater amounts of DαAA (ejected at 40–150 µA, mean 100 µA for 6–15 min, mean 10 min) had no obvious effect on neuronal responses evoked by noxious or non-noxious peripheral stimuli (3 cells in lamina IV and 2 cells in lamina V).

Discussion. The present results suggest the DαAA selectively reduces the response of neurones in the cat dorsal horn to discrete peripheral stimulation. Thus, on the same cells, only the responses to non-noxious stimuli were affected whereas those to noxious peripheral stimuli were unchanged.

Hitherto, the excitatory amino-acid transmitter l-glutamate has been considered to be a putative neurotransmitter in primary sensory pathways[8,9]. The pharmacological selectivity of DαAA (see introduction) and our present observations support the notion that an excitant amino-acid (probably glutamate or aspartate-like) is used as a neurotransmitter in some sensory spinal pathways. On the other hand the polypeptide, substance P is considered to be the primary sensory transmitter on dorsal horn cells receiving nociceptive peripheral input. Thus nociceptive neurones may be preferentially excited by substance P and substance P facilitates the activation of such neurones by noxious peripheral stimulation[7,10,11]. The failure of DαAA to affect noxious responses when ejected into the immediate vicinity of dorsal horn neurones or into the substantia gelatinosa (where substance P positive axons terminate[12]) is in keeping with its lack of interaction with substance P on 2 cells in the present experiments and in in vitro experiments[3,4] and with



Ratemeter record illustrating the effects of Dα-aminoadipate (DAA) on excitatory responses of a lamina IV (upper record) and lamina V (lower record) dorsal horn neurone induced by L-glutamate (GLUT 60^{nA}) and noxious radiant heat (H) and non-noxious air jet (A) stimuli applied to the ipsilateral hind foot. The lines above and below each record indicate the electrophoretic drug ejections or peripheral stimuli, and the lengths of the lines correspond to the duration of ejection or stimulation. The upper record shows the selective and reversible reduction of L-glutamate induced excitation by DAA 40–100 nA. The lower record illustrates selective reduction by DAA 80 nA of synaptic responses to peripheral non-noxious stimulation.

the hypothesis that spinal nociceptive pathways use a transmitter other than an excitatory amino-acid. In conclusion therefore, DαAA offers a useful pharmacological tool to elucidate potential amino-acid mediated synaptic excitation in the central nervous system.

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Modification des équilibres acido-basiques par le jéjunum de rat, in vitro

Acid-base changes across the rat jejunum, in vitro

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Summary. Everted sacs of rat jejunum are able to adjust to near 6.5 any solution whose pH is situated between 5.5 and 9.5. Beyond these limits the adjustment becomes incomplete. Changes in PCO_2 and total CO_2 associated with changes in mucosal pH-values suggest that bicarbonate or CO_2-movements toward the jejunal lumen involved in this adjustment process are predominant.

In vivo, le pH du jéjunum varie selon les espèces; c'est ainsi qu'il se situe, en dehors des périodes prandiales, aux environs de 7,2 chez le lapin[1], 7,0 chez le chien[2], 7,4 chez l'homme[3] et 6,5 chez le rat[4,5]. De nombreux auteurs ont cherché dans quelle mesure les portions proximales de l'intestin étaient susceptibles d'ajuster à des valeurs proches de celles observées chez l'animal entier, le pH de solutions provenant soit directement de l'estomac[6,7], soit perfusées dans des anses isolées (type Thiry[8] ou Thiry-Vella[9]). Meyer et coll.[10], Dorricott et coll.[8], Dolisi et coll.[11] ont montré que le jéjunum de chien est capable, in vivo, d'amener rapidement à des valeurs proches de la neutralité des solutions luminales dont le pH est compris entre 1,7 et 11,3. Cependant, cette technique chez l'animal entier, ne permet pas de mettre en évidence les mécanismes précis de cet ajustement qui participe à la régulation du milieu intérieur. C'est pourquoi, nous nous proposons d'entreprendre une étude parallèle de ces phénomènes, in vitro, en cherchant, dans un premier temps, si le jéjunum de rat présente les mêmes possibilités d'adaptation. Nous avons utilisé la technique du «sac retourné» de Wilson et Wiseman[12], cette méthode ayant permis de mesurer, entre autres, des transports de substances très diverses, telles que le glucose et les acides aminés[13], le sodium[14], l'eau[15].

Le but de ce présent travail est de tester les capacités d'ajustement du jéjunum de rat en mesurant simultanément, d'une part, la pression partielle de gaz carbonique et le contenu total en CO_2, d'autre part, les mouvements des ions monovalents principaux (Na^+ et Cl^-) qui traversent la paroi intestinale.

Matériel et méthodes. Des rats mâles, Wistar, pesant 200–250 g, ayant accès à la nourriture et à l'eau ad libitum, sont anesthésiés à l'éther. Un segment de jéjunum d'environ 15 cm de long est prélevé et directement transféré dans une solution saline (selon Krebs, modifié: NaCl 117 mM; $MgSO_4$, $7H_2O$ 1,2 mM; KCl 5,7 mM; NaH_2PO_4, $2H_2O$ 1,2 mM; $CaCl_2$ 2,5 mM; $NaHCO_3$ 12 mM; glucose 5 mM), à pH 7,4 maintenue à 37 °C et gazée par de l'air. Le contenu intestinal est vidangé par le passage de 20 ml de solution saline. Le fragment est alors retourné et coupé en 3 parties. Chaque portion est montée sur une canule de polyéthylène afin de constituer un sac que l'on remplit de la solution de Krebs. Celle-ci contient, en outre, dans certaines expériences, un marqueur radioactif, le ^{36}Cl (Biochemical Centre, Amersham, Angleterre) destiné à mesurer le flux unidirectionnel séreux-muqueux de cet ion. L'ensemble «canule + intestin» est introduit dans un tube renfermant 5 ml d'une des solutions mucosales à tester. Celles-ci contiennent au départ 105 mM de NaCl et 75 mM de glucose; les différents pH sont obtenus en ajoutant en quantités convenables soit du glycocolle et de la soude (pour les pH compris entre 8,0 et 11,0), soit de l'acide citrique et du phosphate disodique (pour les pH compris entre 2,2 et 8,0) soit de l'acide chlorhydrique 0,1 N (pour les pH inférieurs à 2,2). L'osmolarité des milieux muqueux et séreux est maintenue proche de 310 mOsm afin d'éviter tout gradient de pression osmotique. Le tube est ensuite hermétiquement fermé autour de la canule supportant le sac. La préparation est incubée pendant 60 min à 37 °C. A la fin de l'incubation, on mesure dans la solution baignant la muqueuse, le pH (pH-mètre Orion Resarch 601), les concentrations de sodium (spectrophotomètre d'absorption atomique Perkin-Elmer, 103) et de chlore (chloridomètre radiometer), l'apparition de l'isotope (compteur à scintillation liquide, Intertechnique, SL 32), la pression partielle en CO_2 (Corning pH-blood gazes 165) ainsi que le contenu total en CO_2 par la méthode manométrique de Van Slyke. Vérifiées systématiquement sur un échantillon témoin, les variations du pH des solutions utilisées ne dépassent jamais 0,05 unité pH à la fin des 60 min d'incubation.

Résultats. Ajustement du pH muqueux final. Comme on le voit sur la figure, A, lorsque le pH muqueux initial est compris entre 5,5 et 9,5, le pH luminal obtenu en fin d'incubation se situe vers 6,5. On n'obtient pas de résultats significativement différents si le côté séreux est gazé soit par de l'air soit par un mélange O_2:CO_2 (95:5%). En