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Effects of a selective 5-$HT_{1B/1D}$ receptor agonist on spinal and trigeminal reflexes in the anaesthetized rabbit

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1 The effects of the 5-$HT_{1B/1D}$ receptor agonist L-741,604 on a trigeminally-mediated (jaw depressor) reflex and a spinally-mediated (flexion withdrawal) reflex have been compared between spinalized and intact, anaesthetized rabbits.

2 L-741,604 depressed the jaw depressor reflex dose-dependently in all animals, to a median of 5% (inter-quartile range, IQR, 3–28%, $n=18$) of pre-drug levels after a cumulative dose of 3.1 μmol kg^{-1} i.v. This effect was reversed by the 5-$HT_{1B/1D}$ antagonist GR 127,935 (1–2 μmol kg^{-1} i.v.).

3 The flexion withdrawal reflex was depressed by L-741,604 in non-spinalized animals, to a median of 22% (IQR 10–36%, $n=10$) of pre-drug levels after the highest dose, an action that was reversed by GR 127,935.

4 In spinalized rabbits, L-741,604 up to 0.3 μmol kg^{-1} i.v. cumulative increased the flexion reflex to a median of 189% (IQR 169–198%, $n=8$) of pre-drug controls. With higher doses the reflex decreased, so that after 3.1 μmol kg^{-1} it was 75% (IQR 55–96%) of pre-drug levels. Subsequent GR 127,935 increased reflexes to a median of 180% (IQR 136–219%) of controls.

5 L-741,604 increased arterial blood pressure and decreased heart rate in both preparations, effects that were reversed by GR 127,935.

6 Thus, when the spinal cord was intact L-741,604 inhibited spinal and trigeminal reflexes in the same way. Although spinalization enabled a non-5-$HT_{1B/1D}$-mediated excitatory effect of L-741,604 on spinal reflexes, there was a clear inhibitory effect of the drug at high doses. These data suggest that L-741,604 inhibits spinal reflexes by increasing descending inhibition and by a direct action in the cord. The same processes could apply to inhibition of trigeminally-mediated events.
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Keywords: Migraine; nociception; trigeminal reflex; spinal reflex; withdrawal reflex; serotonin; 5-$HT_{1B/1D}$ receptors

Abbreviations: FWR, flexion withdrawal reflex; GR 127,935, 2′-methyl-4′-(5-methyl-(1,2,4) oxadiazol-3-yl)-biphenyl-4-carboxylic acid (4-methoxy-3-(4-methyl-piperazin-1-yl-phenyl)-amide.HCl; IQR, inter-quartile range; JDR, jaw depressor reflex; L-741,604, N,N-dimethyl-2-[5-1(1,2,4-triazol-4yl)-1-H-indol-3-yl]ethylamine; TA, tibialis anterior

Introduction

Selective agonists for 5-$HT_{1B/1D}$ receptors have become the front line treatment for migraine headache. Their efficacy appears to hinge on two actions: constriction of intracranial blood vessels, and inhibition of the trigeminal nuclear neurones that relay nociceptive inputs from the dura to higher centres. It is not certain which, if either, of these two effects is the more important (Goadsby, 1998). It is usual for drugs that influence caudal trigeminal neurones to have the similar effects in the spinal cord. However, this may not be true for 5-$HT_{1B/1D}$ receptor agonists, which are always found to inhibit the responses of trigeminal nociceptive neurones (Goadsby & Edvinsson, 1994; Hoskin *et al*., 1996; Goadsby & Hoskin, 1996; 1998; Cumberbatch *et al*., 1997; 1998a,b; 1999; Storer & Goadsby, 1997), but have weak or undetectable effects against spinal dorsal horn cells (Gjerstad *et al*., 1997; Cumberbatch *et al*., 1998b).

In a recent study from this laboratory, it was reported that the potent 5-$HT_{1B/1D}$-receptor agonists L-694,247 and L-741,604 increased a spinal withdrawal reflex in the decerebrated, spinalized rabbit (Ogilvie *et al*., 1999). This effect was found not to be reversed by the 5-$HT_{1B/1D}$ antagonist GR 127,935 and, as there was clear evidence from cardiovascular changes observed in the experiments that the doses of agonist used had activated the appropriate receptors, it was concluded that 5-$HT_{1B/1D}$-receptors have little or no role to play in the modulation of spinal withdrawal reflexes. We took this as further evidence of a differential action of triptan-type drugs in trigeminal *versus* spinal systems. However, there are few reports on the effects of 5-$HT_{1B/1D}$-receptor agonists on trigeminally-mediated reflexes. Thus, the purpose of the present study was to investigate the actions of L-741,604 against trigeminal and spinal reflexes recorded in the same animals. In these experiments we have chosen to investigate the jaw depressor reflex (JDR), evoked by electrical stimulation of the tongue and recorded from the digastric muscle, and a flexion withdrawal reflex (FWR) evoked by electrical stimulation of the toes and recorded from the ankle flexor muscle tibialis anterior (TA). Both are nocifensive reflexes and are commonly used as simple indicators of nociceptive processing in trigeminal and spinal systems, respectively. In order to avoid the damage to the dura mater and superior sagittal sinus that accompanies decerebration, the present study has been performed in pentobarbitone-anaesthetized rabbits. A preliminary account of this work has been published (Jenkins *et al*., 2000).

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Methods

Experiments were performed on 18 rabbits of various strains and either sex, weighing from 1.7–4.1 kg.

Basic preparation

Rabbits were sedated with ketamine hydrochloride (Ketaset, Willows Francis Veterinary, 50 mg, i.m.). Anaesthesia was induced with pentobarbitone sodium (Sagatal, Rhone, May and Baker Animal Health Ltd, average dose 43 mg kg^{-1}, i.v.), and maintained with the same agent, given as a slow i.v. infusion with a mean rate of 11.7 mg kg^{-1} h^{-1} (anaesthesia was monitored using blink reflex evoked by gently touching the cornea, and the infusion rate adjusted accordingly). Rectal temperature was monitored using a thermostatically controlled heating blanket, which maintained the core body temperature at $38 \pm 0.5^{\circ}$C.

The trachea was cannulated in order to provide a free airway and allow subsequent artificial ventilation. The left carotid artery was cannulated to allow measurement of arterial blood pressure and the left jugular vein was cannulated and used for the infusion of anaesthetic. The left brachiocephalic vein was also cannulated for the delivery of other drugs.

Rabbits were ventilated on room air supplemented with oxygen using a Starling ideal pump. End tidal CO_2 percentage was measured at intervals to ensure adequate ventilation. ECG was recorded from paired sub-cutaneous electrodes placed either side of the chest to provide a record of heart rate.

Preparation for spinalization

At this point eight of the rabbits were spinalized. An incision was made over the thoracic 12 (T12) and lumbar 1 (L1) region of the spinal cord. The muscle between the two vertebrae and the bone of L1 (overlying the L1 segment) was removed, and the spinal cord divided by suction after an injection of 100 μl of lignocaine. Cotton wool soaked in Ringer's solution was used to pad out the hole and the wound was closed.

Stimulation and recording

In all animals, stimulus–response relationships were determined for the jaw depressor reflex (JDR) and the hind limb flexion withdrawal reflex (FWR). The JDR was evoked through paired stainless steel needle electrodes inserted into the tongue, and recorded *via* two varnish-insulated copper wire electrodes inserted into the left digastric muscle. The FWR was activated *via* stimulation of the skin of the plantar surface at the base of the toes, again through paired stainless steel needle electrodes, and recorded from the tibialis anterior (TA) muscle of the left hind limb using paired copper electrodes.

Constant current stimuli of 1 ms duration were applied to the tongue and the toes and stimulus–response curves were constructed. Thresholds (T) for each stimulation site were obtained by increasing the current until the first signs of a consistent response could be visualized. The current was then increased in predetermined increments (see below) and the response measured as described below. The stimulus used in the rest of the experiment was one that gave approximately two-thirds of the largest response found in this part of the experiment.

Rabbits were left for 30 min to adjust to the conditions, after which time the experiment was started. For control data, tongue and toes were stimulated alternately (stimulus range for FWR 3.5–47 mA; and for the JDR 0.4–7 mA, see Results), every 2 min. Each stimulus session consisted of eight shocks given at 1 Hz. This was carried out for a period of at least 30 min until reflex responses were stable. The eight reflex responses from a stimulus session were averaged and integrated with respect to time, to give a measure of reflex excitability (area under the averaged signal). Signals were amplified to between 1000 and 10,000 times, filtered between 1 Hz and 5 kHz, and digitized using a Cambridge Electronic Design 1401 Interface connected to a PC running SIGAVG Version 6.3. Signals were full-wave rectified before measurement.

Experimental protocol – the effect of a 5-HT$_{1B/1D}$ agonist on trigeminal and spinal reflexes

Following the control period, L-741,604 was given i.v. at intervals of 24 min and at increasing doses of 0.03, 0.06, 0.22, 0.62 and 2.15 μmol kg^{-1} to give a cumulative total of 3.08 μmol kg^{-1}. After the final dose of L-741,604, a single dose of GR 127,935 (1–2 μmol kg^{-1}) was given i.v. and the reflexes recorded until the response reached a maximum level (between 30–60 min).

Drugs

L-741,604 (N,N-dimethyl-2-[5-1(1,2,4-triazol-4yl)-1-H-indol-3-yl]ethylamine) a gift of Dr M. Cumberbatch, Merck, Sharp and Dohme Neuroscience Research, was dissolved in Ringer's solution to a concentration of 6.2 mM and serial 10 fold dilutions were made in the same solvent. GR 127,935 (2′-methyl-4′-(5-methyl-(1,2,4) oxadiazol-3-yl)-biphenyl-4-carboxylic acid (4-methoxy-3-(4-methyl-piperazin-1-yl-phenyl)-amide.HCl) a gift of Glaxo Wellcome Research, was dissolved in dimethyl sulphoxide (DMSO) and subsequently diluted to a strength of 4.0 mM in Ringer's solution (final DMSO concentration 0.5%).

The composition of Ringer's solution used was (mM): NaCl 142, $NaHCO_3$ 2, KCl 4, $CaCl_2$ 2 and $MgCl_2$ 0.05.

Statistical analysis

Reflexes are expressed as a percentage of the mean of the last six values recorded immediately before the first dose of L-741,604 was given. Reflex data were not normally distributed and are expressed as medians with inter-quartile ranges (IQRs). Dose-response data were analysed using Friedman's ANOVA on ranks followed by Dunn's test. Other comparisons were by Wilcoxon matched pairs test or Mann-Whitney *U*-test as appropriate. Cardiovascular data were suitable for parametric analysis and are expressed as mean values ± s.e.mean. They have been analysed using repeated measures ANOVA and paired and unpaired *t*-tests as appropriate. All *P* values are 2-tailed, and were calculated using Instat 3 program from GraphPad software.

Results

Stimulus–response relationships

The jaw depressor reflex The median threshold stimuli for evoking reflexes in the digastric muscle were 0.9 mA (IQR 0.5–1.4 mA) and 1.5 mA (IQR 1.0–1.7 mA) in spinalized ($n=8$) and non-spinalized ($n=10$) rabbits respectively. These values were not significantly different from each other (Mann Whitney test, $P=0.5$). The JDR increased sharply with stimulus intensity above threshold (T, Figure 1), so that

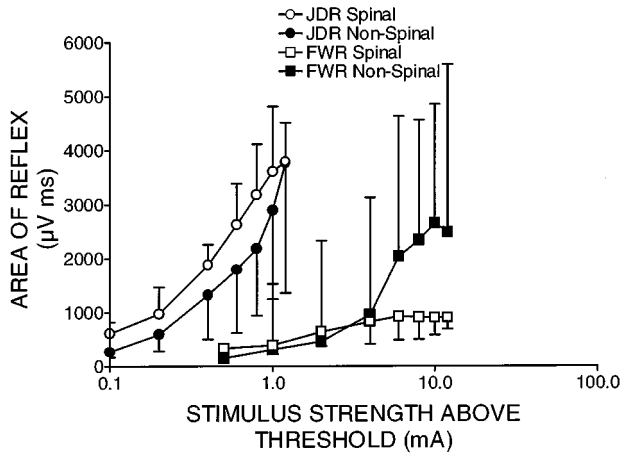

Figure 1 Stimulus–response relationships for the jaw depressor reflex (JDR) and flexion withdrawal reflex (FWR) in spinalized and non-spinalized rabbits. Each point is a median value and the vertical bars indicate first or third quartiles.

maximal responses were achieved with stimuli between 1 and 1.2 mA greater than T in spinalized animals. The regime for non-spinalized animals was based on that found to obtain in spinal preparations and the maximum stimulus used did not exceed T + 1.2 mA. The stimulus–response curves for this reflex are statistically indistinguishable between spinalized and non-spinalized preparations (Mann Whitney tests, $P > 0.1$ at each stimulus strength).

For this reflex, and the FWR (see below), the stimulus used to evoke reflexes in the rest of the experiment was set to give approximately 66% of the largest reflex obtained in constructing the stimulus–response curves. Thus, the median stimulus intensities used to evoke the JDR were 0.6 mA (IQR 0.4–0.7 mA) and 0.9 mA (IQR 0.7–1.0 mA) above threshold for the spinalized and non-spinalized animals, respectively. Again, these values were not significantly different.

The flexion withdrawal reflex in tibialis anterior The median threshold for eliciting this reflex in spinalized animals was 0.9 mA (IQR 0.5–1.5 mA), whereas in non-spinalized preparations it was not quite significantly higher (Mann Whitney test, $P = 0.08$) at 2.5 mA (IQR 1.0–3.4 mA). In neither preparation was the threshold for the FWR significantly different from that for the JDR (Wilcoxon tests, $P = 0.06$ for non-spinals and 0.7 for spinals).

The stimulus–response curves for the FWR were rather flatter than for the JDR (Figure 1), so that maximal responses were reached with stimuli between 6–8 mA above threshold in spinalized rabbits and 10–12 mA greater than T in non-spinalized animals. The median stimulus intensities used to evoke this reflex in the rest of the experiment were 6.6 mA (IQR 6–10 mA) and 9.0 mA (IQR 7–12 mA) greater than threshold for spinal and non-spinal preparations respectively.

Effects of L-741,604 on the JDR

Spinalized preparations L-741,604 induced a significant (Friedman's ANOVA, $P < 0.0001$, $n = 8$), dose-related decrease in JDR responses, to a median of 5% (IQR 3–13%) of pre-drug controls after the final dose. The minimum effective dose was 0.9 μmol kg^{-1} cumulative (Dunn's post-test, $P < 0.05$). After GR 127,935, the reflex was restored to a median of 71% (IQR 50–106%) of pre-drug values. This was significantly greater than the post-L-741,604 level (Wilcoxon test, $P = 0.008$), but was not different from the pre-drug control (Wilcoxon test, $P = 0.2$).

Non-spinalized preparations L-741,604 induced a highly significant (Friedman's ANOVA, $P = < 0.0001$, $n = 10$), dose-related decrease in JDR, to a median of 9% (IQR 6–49%) of pre-drug controls after the final dose (Figure 2). The minimum effective dose was 0.9 μmol kg^{-1} cumulative (Dunn's post-test, $P < 0.01$). GR 127,935 antagonized the effect of L-741,604, so that after this drug, the JDR was a median of 85% (IQR 40–157%) of pre-L-741,604 levels (Figure 2), significantly greater than after the highest dose of the agonist (Wilcoxon test, $P = 0.002$), but not significantly different from pre-drug values (Wilcoxon test, $P = 0.7$).

There were no significant differences in the effects of these drugs between spinalized and non-spinalized preparations. If the two groups of animals are combined, the overall effect of L-741,604 was to reduce the JDR to a median of 5% (IQR 3–28%) of pre-drug levels, with GR 127,935 returning the reflex to 66% (IQR 38–107%) of controls ($n = 18$). The larger group gives a lowest effective dose of L-741,604 as 0.3 μmol kg^{-1} cumulative.

Effects of L-741,604 on the FWR in tibialis anterior

Spinalized preparations Low doses (up to 0.3 μmol kg^{-1} cumulative) of the 5-HT$_{1B/1D}$ receptor agonist significantly (Friedman's ANOVA, $P < 0.0001$) enhanced the FWR, to a median of 189% (IQR 169–198%) of pre-drug values, after a cumulative dose of 0.09 μmol kg^{-1} (Figure 3). At higher doses, the reflex declined in size so that after the final dose of L-741,604, the TA reflex response was a median of 75% (IQR 55–96%) of control levels, although Dunn's post-test failed to show this as significantly different from control ($P > 0.05$). GR 127,935 enhanced reflexes to a level (median 180%, IQR 136–219%) that was greater than the pre-drug value (Wilcoxon, $P = 0.02$) but not different from the peak increase seen with low doses of L-741,604 (Wilcoxon, $P = 0.8$, Figure 3).

There were significant differences between the effects of L-741,604 on the JDR and FWR in these preparations at all doses used (Wilcoxon tests, $P < 0.02$).

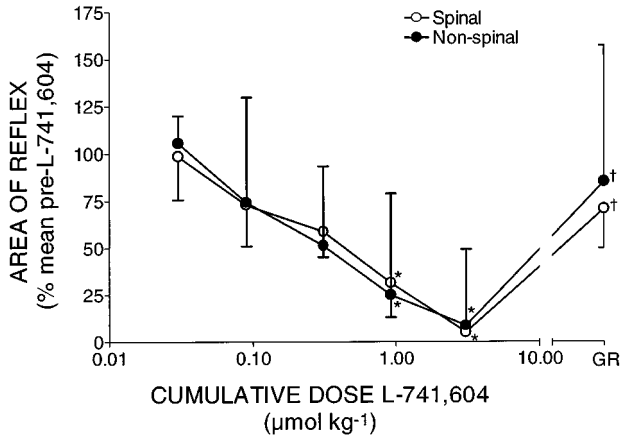

Figure 2 The effects of L-741,604 and subsequent GR 127,935 (GR, 1–2 μmol kg^{-1}) on the JDR evoked by stimulation of the tongue in spinalized and non-spinalized rabbits. Each point is a median value and the vertical lines indicate first or third quartiles. *Indicates a value significantly less than pre-drug control; †Indicates a value significantly greater than the post-L-741,604 level but not significantly different from control.

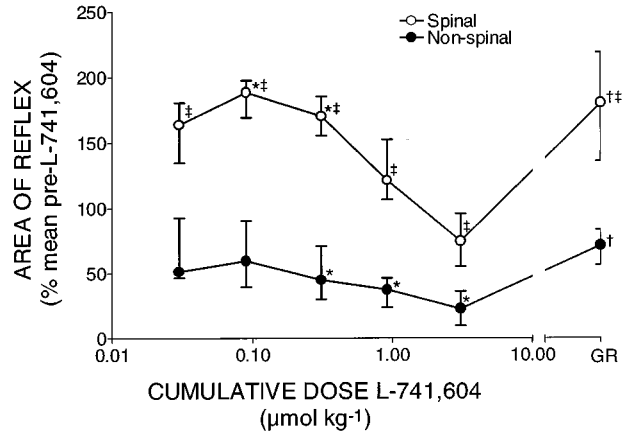

Figure 3 The effects of L-741,604 and subsequent GR 127,935 (GR, 1–2 μmol kg^{-1}) on the FWR evoked by stimulation of the toes in spinalized and non-spinalized rabbits. Each point is a median value and the vertical lines indicate inter-quartile ranges. *Indicates a value significantly different from pre-drug control; ‡Indicates a significant difference between spinalized and non-spinalized preparations; †Indicates a value significantly greater than the post-L-741,604 level.

Non-spinalized preparations In these animals, L-741,604 induced a significant (Friedman's ANOVA, $P<0.0001$, $n=10$), dose-related decrease in hind limb withdrawal responses, to a median of 22% (IQR 10–36%) of pre-drug controls after the final dose (Figure 3). The minimum effective dose was 0.3 μmol kg^{-1} cumulative (Dunn's post-test, $P<0.01$). This effect was reversed by GR 127,935 (1–2 μmol kg^{-1}), so that after the antagonist the FWR was a median of 71% (IQR 56–84%) of pre-L-741,604 levels (Figure 1). This was significantly larger than after the final dose of L-741,604 (Wilcoxon test, $P=0.002$), but was not significantly different from the pre-drug controls (Wilcoxon test, $P=0.6$).

There were significant differences in the effects of L-741,604 and GR 127,935 on the FWR between spinalized and non-spinalized preparations at all doses tested (Mann-Whitney tests, $P<0.03$).

Cardiovascular effects

Spinalized preparations In these animals, mean pre-drug arterial blood pressure and heart rate were 68±5 mmHg and 281±8 beats min^{-1} respectively. L-741,604 induced a dose-dependent increase in mean arterial pressure, by a mean of 37±3 mmHg over pre-drug values, a highly significant change (repeated measures ANOVA, $P<0.0001$, Figure 4). The minimum effective dose was 0.03 μmol kg^{-1} (Tukey post-test, $P<0.05$). Heart rate decreased as blood pressure increased, so that after the final dose of L-741,604, it was a mean of 22±6 beats min^{-1} lower than the pre-drug value ($P<0.0001$, repeated measures ANOVA). Again, the lowest effective dose was 0.03 μmol kg^{-1}. GR 127,935 antagonized both effects, so that after this drug mean arterial pressure was 16±4 mmHg above, and heart rate 2±5 beats min^{-1} below, pre-drug levels. In both cases the change relative to post-L-741,604 values was significant (paired t-test, $P<0.01$), but whereas the post-GR 127,935 heart rate was not significantly different from controls (paired t-test, $P=0.6$), the blood pressure remained significantly elevated (paired t-test, $P=0.01$).

Non-spinalized preparations The mean control values for arterial pressure and heart rate in these animals were 96±3 mmHg and 290±10 beats min^{-1}. Blood pressure was predictably higher than in spinalized rabbits (t-test, $P<0.0001$). L-741,604 increased blood pressure and decreased heart rate as it did in spinalized preparations (Figure 4), so that after the final dose, arterial pressure was an average of 27±6 mmHg higher than control and heart rate 46±5 beats min^{-1} lower. Both changes were highly significant (repeated measures ANOVA, $P<0.0001$). The lowest effective dose for changing heart rate was 0.03 μmol kg^{-1}, whereas that for blood pressure was 0.3 μmol kg^{-1} cumulative (Tukey tests, $P<0.05$). The changes in arterial blood pressure were significantly less than those seen in spinalized rabbits at 0.03, 0.09 and 3.1 μmol kg^{-1} cumulative doses (t-tests, $P<0.02$), and almost significant at the other doses, whereas the fall in heart rate was significantly greater for non-spinals only after the 3.1 μmol kg^{-1} cumulative dose (t-test, $P<0.05$).

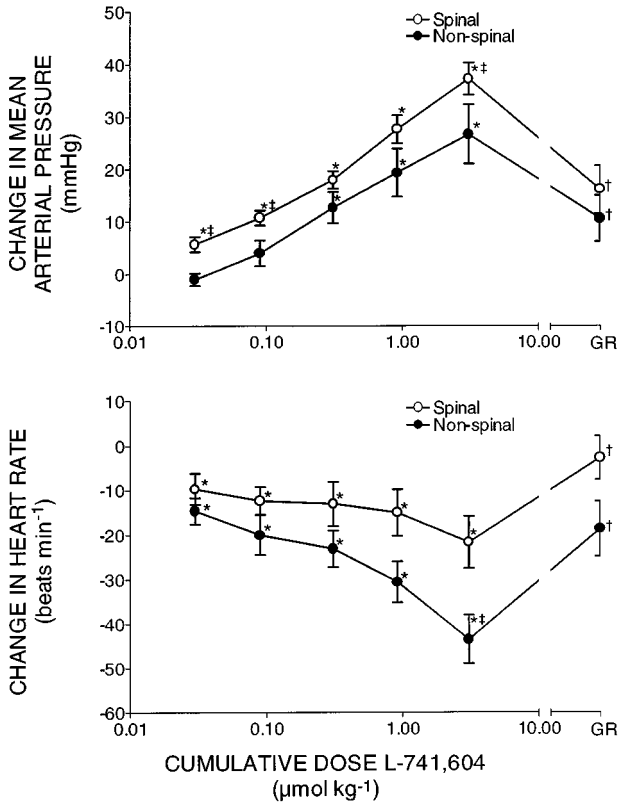

Figure 4 Cardiovascular changes induced by L-741,604 and subsequent GR 127,935 (GR, 1–2 μmol kg^{-1}) in spinalized and non-spinalized rabbits. Top panel: Changes in arterial blood pressure: Lower panel: Changes in heart rate. Each point is a mean±s.e.mean. *Indicates a value significantly different from pre-drug control; ‡Indicates a significant difference between spinalized and non-spinalized preparations; †Indicates a value significantly greater than the post-L-741,604 level.

GR 127,935 again antagonized the cardiovascular actions of L-741,604. After the antagonist, blood pressure was 11±4 mmHg higher, and heart rate 19±6 beats min^{-1} lower than controls. These values were both significantly different from the post-L-741,604 levels (paired t-tests, $P<0.0001$), but also remained significantly different from pre-drug values (paired t-tests, $P<0.0001$).

Discussion

In the present study reflexes were evoked by electrical stimulation of peripheral structures by percutaneous electrodes, effectively bypassing peripheral receptor mechanisms. Thus, any effects of 5-HT$_{1B/1D}$ receptor agonists and

antagonists on reflex responses were mediated in the central nervous system, even though the drugs were given systemically. For animals with a spinal section, drug-induced changes in the FWR would have been due to actions in the spinal cord itself. However, in non-spinalized rabbits it is not possible to say in which part of the CNS any effects were mediated, other than by comparison with results from spinalized preparations.

Trigeminal versus *spinal actions of 5-HT$_{1B/1D}$ receptor agonists*

The objective of the present experiments was to investigate the possibility that agonists of 5-HT$_{1B/1D}$ receptors have a selective inhibitory action against trigeminal as opposed to spinal nocifensive reflexes. The results show that the answer to this question depends on whether or not the spinal cord is intact, and do not support the idea of a fundamental difference between the effects of triptan-type drugs on trigeminal and spinal systems.

The jaw depressor reflex, a trigeminally-mediated nocifensive reflex

The jaw depressor (often called the 'jaw-opening') reflex is a nocifensive response evoked by intense or irritating intra-oral stimuli that has often been used as an indicator of trigeminal nociceptive excitability (Mason *et al*., 1985). However, it involves the mandibular division of the trigeminal nerve rather than the ophthalmic area that is associated with migraine, and its central pathway involves the oral part of the trigeminal nuclear complex (Kidokoro *et al*., 1968), not the caudal portion that is most often associated with nociceptive processing (Sessle, 1996; but see Campbell *et al*., 1985). Furthermore, the stimuli required to evoke the reflex in the present study were rather low, and the stimulus-response curve very steep, particularly when compared to that for the hind limb FWR. We account for the latter observation by the very high density of sensory receptors in the tongue compared to the foot, but it was by no means certain at the outset of this project that drugs which inhibit responses to dural nociceptors would also suppress this reflex.

In the event, the effects of L-741,604 on the JDR were exactly what would be predicted from the previous data obtained with this and related drugs in depressing trigeminal nociceptive responses (Hoskin *et al*., 1996; Goadsby & Hoskin, 1996; Cumberbatch *et al*., 1997; 1999). Inhibition of the JDR was achieved with a reasonable dose of the agonist (<1 mg kg^{-1}) and was reversed by GR 127,935, thus firmly indicating the involvement of 5-HT$_{1B/1D}$ receptors in this action. As noted above, this effect must have been mediated centrally as the reflex was evoked by electrical stimulation. Furthermore, the behaviour of this reflex and its responses to drugs were predictably unaffected by low spinal section, showing that changes in the ascending flow of sensory information did not significantly affect transmission through the JDR pathway.

The hind limb flexion withdrawal reflex in tibialis anterior, a spinally-mediated nocifensive reflex

Spinalized preparations L-741,604 had different effects on this reflex dependent on whether or not the spinal cord was intact. In spinalized animals, the FWR increased with doses up to 0.3 μmol kg^{-1} in exactly the fashion previously described for the sural-medial gastrocnemius reflex of spinalized, decerebrated rabbits (Ogilvie *et al*., 1999), even though we recorded a different reflex and used anaesthetized rather than decerebrated, unanaesthetized animals in the present study. Increasing the dose beyond this level caused the reflex to decline in size, so that after the highest dose, TA responses were just smaller than the pre-drug control. Only this inhibitory effect was reversed by GR 127,935, as the reflex was restored to its facilitated state after the 5-HT$_{1B/1D}$ antagonist.

What these data show is that the enhancement of reflexes by L-741,604 is not mediated by 5-HT$_{1B/1D}$ receptors, as observed previously (Ogilvie *et al*., 1999), but that high doses of the drug produce inhibition in the spinal cord that is effected through these sites. It is evident that our earlier conclusion that 5-HT$_{1B/1D}$ receptors play little or no role in modulating spinal reflexes can no longer be sustained. Only marginal signs of this inhibitory action were apparent in our earlier experiments, even though the cardiovascular effects of L-741,604 showed quite clearly that a sufficient dose of the drug had been given to provide substantial activation of 5-HT$_{1B/1D}$ receptors. This explains why we did not use doses higher than 0.3 μmol kg^{-1} in those studies (Ogilvie *et al*., 1999). L-741,604 would appear to be more potent at this non-5-HT$_{1B/1D}$ site, which we believe may be a 5-HT$_7$ receptor or something similar, than at its 'preferred' receptor. A more complete characterization of this site is now required. Understanding the nature of the receptor that causes enhancement of reflexes, and the interactions of triptan-like drugs with it, could help to explain some of the side-effects of these agents (Goadsby, 1998; Diener & Limmroth, 1999).

Non-spinalized preparations In contrast to its effects in spinalized rabbits, L-741,604 caused only inhibition of spinal reflexes in non-spinalized animals, so that the effects of the drug on the FWR were indistinguishable from those seen for the JDR. Although some signs of facilitation were seen in a few animals, generally speaking the enhancement of reflexes that dominated with low doses of L-741,604 in spinalized rabbits, was absent. The differences between spinalized and non-spinalized rabbits must relate to activity in descending pathways, which we hypothesize could be due to two sorts of effect: (i) activation of 5-HT$_{1B/1D}$ receptors in the brain stem stimulates descending inhibitory systems that are powerful enough to overcome the facilitatory effects seen in spinalized animals; or (ii) descending pathways somehow disable these facilitatory effects so that only the 5-HT$_{1B/1D}$ receptor-mediated inhibition is seen. We cannot yet discriminate between these options.

If descending inhibitory pathways are important in determining the central actions of 5-HT$_{1B/1D}$ receptor agonists, they may be an important part of the anti-migraine action of these compounds. Several lines of evidence suggest that migraine headache may result from a failure of central inhibitory processes (Sicuteri, 1976; Lance, 1992; Diener, 1997; Hargreaves & Shepheard, 1999), whilst those areas of the brain stem known to have inhibitory effects on spinal nociceptive neurones, such as the locus coeruleus, periaqueductal grey matter and rostral ventromedial medulla are known also to inhibit trigeminal pain processing (Sessle & Hu, 1981; Sessle *et al*., 1981). It is difficult to isolate the JDR pathway from these influences (there is no trigeminal equivalent of spinalization), so the way to test this idea may be to study the effects of microinjections of 5-HT$_{1B/1D}$ agonists into the appropriate brain stem regions. While activation of descending inhibition may contribute to the central inhibitory effects of triptans, it cannot be the only effect, as direct application of these drugs

to trigeminal neurones by iontophoresis results in inhibition (Storer & Goadsby, 1997).

The depression of spinal withdrawal reflexes by a drug is usually a sign that the agent in question is analgesic. However, what little evidence there is suggests that triptan-like drugs are not effective in anything other than head pains (Skingle *et al*., 1990; Dao *et al*., 1995; Roberts-Thomson *et al*., 1996; Connor *et al*., 1997). Perhaps it is time that this question was revisited. It has recently been shown that the rat Htr1b gene, which codes for the 5-HT$_{1B}$ receptor, has a central role in determining differences in the analgesic potency of morphine in in-bred strains of mice (Hain *et al*., 1999).

Cardiovascular actions of L-741,604

The changes in cardiovascular parameters seen with L-741,604 were the same as those described in our earlier study (Ogilvie *et al*., 1999): a pressor effect, presumably caused by vasoconstriction, accompanied by a fall in heart rate, most of which we believe to be baroreceptor reflex-mediated. However, it should be noted that in non-spinalized animals heart rate fell at a lower dose than blood pressure increased, suggesting that there may be a direct bradycardic action of 5-HT$_{1B/1D}$ receptor activation in rabbit. The other differences between spinalized and non-spinalized animals can be explained by the higher resting blood pressure in the former preparations.

Conclusions

The present data do not support the view that there are fundamental differences in the effects of 5-HT$_{1B/1D}$ receptor activation between spinal and trigeminal systems. Rather, they show that the 5-HT$_{1B/1D}$ receptor-mediated inhibition caused by L-741,604 was masked by a non-5-HT$_{1B/1D}$ receptor-mediated facilitation of reflexes when descending pathways were interrupted. One interpretation of our data in respect of spinal reflexes is that the primary action of low doses ($\leqslant 0.3$ μmol kg^{-1}) of L-741,604 is to activate descending inhibitory systems, while higher doses ($\geqslant 0.9$ μmol kg^{-1}) have a direct suppressant action on spinal neurones. There is no reason why the same processes could not also apply to the effects of 5-HT$_{1B/1D}$ receptor agonists on the trigeminal system, and we believe that this idea now requires investigation.

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