LETTER

Reply from Aihua Li, Charles C. T. Hindmarch, Eugene E. Nattie and Julian F. R. Paton

The authors appreciate the interest of Silvani *et al.* in our recent publication (Li *et al.* 2013). The key points of our paper are: (1) treating spontaneously hypertensive rats (SHR) with a single dose of a dual orexin receptor antagonist, almorexant, significantly decreased blood pressure for 8 h in wakefulness and sleep during both the light and dark period of the diurnal cycle; (2) in the normotensive control (WKY) rats the same treatment did not significantly affect blood pressure in either wakefulness or sleep in any light cycle. Our experimental design includes: (a) having the rats surgically implanted with EEG, EMG and blood pressure radio-telemetry at least 7 days before testing; (b) placing the rats in a light- and temperature-controlled recording chamber for 12 h with food and water available *ad libitum*; and (c) measuring blood pressure, ECG, EEG and neck EMG before and after administration of a dual orexin receptor antagonist.

Silvani *et al.* express concern, not about our major findings, but about our '. . . claim . . . that the effect of Almorexant on BP did not differ significantly between wakefulness and non-rapid-eye-movement sleep (NREMS) in SHR either in the light (rest) or dark (activity) period of the day.'

They raise four 'issues'. First, our recording protocol '. . .may not have been sensitive enough to detect changes in BP related to sleep states and the day–light cycle. . .'. As stated above, we used radio-telemetry of blood pressure (BP) with long uninterrupted recording periods. Figure 2*B* of our study (Li *et al.* 2013) shows that in normotensive WKY rats, this protocol does detect sleep–wake differences.

In SHR, we reported that mean arterial blood pressure was slightly higher in wakefulness compared with NREM sleep, a difference that reached statistical significance in the light period.

In the reference cited by Silvani *et al.*, Kuo & Yang (2005) reported that 'The WKY had significant changes in arterial pressure . . . with the sleep–wake transitions . . . The sleep-related changes . . . , however, were not as evident in the SHR'. The smaller BP difference (∼5 mmHg) between wakefulness and sleep that we observed in SHRs are in fact within the range reported in SHR by Kuo & Yang (2005).

Second, we did not report the effects of almorexant on sleep structure in SHR rats. Silvani *et al.* suggest that improvement of disrupted sleep in SHR by almorexant could '. . .confound the observed results on BP.' As noted by Silvani *et al.*, we (and others) have reported the effects of almorexant on sleep in non-SHR and we agree with their prediction that SHR may sleep 'better' after almorexant, but this requires robust validation. However, we do not believe that this explains the lowering of BP since Lee *et al.* (2013) demonstrated that intracerebroventricular or intra-rostral ventrolateral medulla blockade of orexin 2 receptors significantly reduced arterial pressure in anaesthetized SHRs but not WKY rats.

Third, Silvani *et al.* state that we '. . .did not indicate whether Almorexant modified the difference in BP between wakefulness and NREM sleep in SHR and WKY rats.' Figure 4*A* and *B* of our paper (Li *et al.* 2013) showed the maximum changes in BP before and after almorexant; the largest decrease of BP after blocking orexin receptors was in wakefulness during the dark period (−37 mmHg) and smallest change in NREM sleep during the light period (−25 mmHg) relative to the pre-treatment baseline. In our experiments the observed difference between day and night, quiet wakefulness and sleep is around 10 mmHg. Further, Silvani *et al.* compare our results to human narcolepsy and animal models thereof in which the absence of orexin has been present for much longer time periods than the orexin receptor blockade induced by using almorexant as we performed. This difference between genetic knockout models and acute, reversible orexin receptor inhibition is a key part of our approach.

Fourth, we did not report the effects of our almorexant treatment on blood pressure in REM sleep. This is true and is an important omission from the paper, which we fully acknowledged.

In summary, we agree with Silvani *et al.* that antagonism of orexin receptors has a significant anti-hypertensive effect in unanaesthetized SHR rats, which suggests that modulation of the orexin system could be a potential target in treating hypertension. We thank Silvani *et al.* for their interest in our work.

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