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Short communication

Combining pindolol and paroxetine in an animal model of chronic antidepressant action—can early onset of action be detected?

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Abstract

The realisation that pindolol may accelerate the effects of some antidepressant drugs in clinical trials has added extra impetus to the search for faster acting antidepressants. Currently, no animal model of depression can identify potential faster acting antidepressant drugs or drug combinations. In this study, we investigate the effects of combining pindolol (2 mg/kg, s.c., bid) with the antidepressant paroxetine (2.5 mg/kg, i.p., bid) in the olfactory bulbectomised rat, an animal model of chronic (but not acute) antidepressant activity. Ambulation scores were measured in separate groups of rats, following 3, 7 and 14 days of treatment. Further, we simultaneously study adaptive changes in 5-HT_{1A} receptor function, utilising alterations in the hypothermic response to the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). Pindolol in combination with paroxetine attenuated the hypothermic effects of 8-OH-DPAT as early as 3 days with a full reversal evident following 7 days, whereas paroxetine alone did so after 14 days only. Likewise, paroxetine alone reversed the olfactory bulbectomy-induced hyperactivity in the open field following 14 days of treatment only, this being the normal time of an 'antidepressant' response in this model. However, the group treated with both paroxetine and pindolol failed to reverse the hyperactive response. This suggests that a factor intrinsic to pindolol antagonises the behavioural effects of paroxetine in the olfactory bulbectomised rat. It also demonstrates that the reversal of this aspect of the olfactory bulbectomy-induced behavioural syndrome is insensitive to the potential faster onset of antidepressant action induced by pindolol. The ability of the combination group to attenuate the hypothermic effects of 8-OH-DPAT much faster further emphasises the role of the 5-HT_{1A} receptor in the mechanism of action of antidepressants and as a target for the development of faster acting antidepressants. However, an animal model sensitive to the effects of any such compound and the actions of pindolol remains elusive.

Keywords: Olfactory bulbectomy; Antidepressant; Onset of action; 8-OH-DPAT-induced hypothermia; 5-HT_{1A} receptor; Paroxetine

1. Introduction

The use of the β-adrenoceptor antagonist pindolol as an adjunct to conventional antidepressant treatment has recently stimulated much interest with claims of its ability to decrease the latency period before clinical effect appears (Artigas et al., 1994; Blier and Bergeron, 1995; Perez et al., 1997; Bakish et al., 1997; Maes et al., 1996 and Tome et al., 1997 but see Dinan and Scott, 1996; Berman et al., 1997). The rationale for this is based on pindolol's antagonism at the somatodendritic 5-HT_{1A} receptor, which is believed to play a pivotal role in the antidepressant response (Artigas et al., 1996).

Various animal models have been developed to detect antidepressant activity of compounds and mimic aspects of the idiopathic disease state (see Willner, 1990). However, despite the variety of models available, none have been refined to detect onset of action. As much attention and resources have been directed toward the need to find faster acting antidepressants (Norman and Leonard, 1994), complementary animal models are needed.

The olfactory bulbectomised rat has been validated as a model of depression over the past 20 years (Kelly et al., 1997). It has the advantage over many other models in that many of its behavioural changes occur following chronic but not acute antidepressant treatment, which correlates well with the typical delayed onset of action seen in the

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clinical setting (Janscar and Leonard, 1984; Kelly et al., 1997).

In this study, we assessed the ability of this model to detect an earlier onset of antidepressant action of pindolol in combination with the selective serotonin reuptake inhibitor paroxetine, which has been seen in the clinic. The attenuation of the bulbectomy-induced hyperactivity in the open field by antidepressants following chronic (usually 14 days) treatment has been one of the most consistently reproducible paradigms of the olfactory bulbectomy syndrome (Van Reizen and Leonard, 1991). In the present study, we have investigated the effects of 3, 7 and 14 days combined pindolol and paroxetine treatment on this response. Concurrently, we assessed the effects of drug treatment on 5-HT_{1A} receptor function at these time points by challenging each rat with the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and measuring the degree of hypothermia obtained. The attenuation of 8-OH-DPAT-induced hypothermia has been shown following chronic antidepressant treatment and also by electroconvulsive shock therapy (Goodwin et al., 1985, 1987). As the antidepressant effects of using pindolol as an adjunct have been suggested to be due to adaptive changes at the 5-HT_{1A} receptor, this paradigm is introduced to complement the behavioural data at each time point.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (300–350 g) were brought into the laboratories and were allowed to acclimatise for 1 week prior to any intervention. The animals were housed four per cage in standard hard bottom polypropylene cages ($45 \times 28 \times 20$ cm), containing wood shavings and with stainless steel lids. The animals had ad-libitum access to food and water. The animals were maintained at a constant temperature (room temperature of $21 + 1$°C) and at a standard lighting conditions (12-h light; 12-h dark, lights on from 0800 to 2000). They were handled daily prior to surgery. All procedures were carried out under the guidelines of the animal welfare committee of the Austin and Repatriation Medical Centre, Heidelberg, Victoria, Australia.

2.2. Olfactory bulbectomy

Bilateral olfactory bulbectomy was performed on rats anaesthetised with a 2.5% w/v 2-2-2 tribromo-ethanol anaesthesia (Aldrich, Sydney) (10 ml/kg), essentially as described by Cairncross et al. (1977). The head was shaved and a midline sagittal incision was made extending at least 1 cm rostral to the bregma. Pressure was applied to ensure that the periosteum on the underlying bone had been penetrated. A burr hole was drilled at points 7 mm anterior to the bregma and 2 mm either side of the midline at a point corresponding to the posterior margin of the orbit of the eye. The olfactory bulbs were removed by suction and the burr holes filled with haemostatic sponge (Spongestan, Johnson and Johnson, Sydney). Tetracycline powder was applied to the wound prior to closure using surgical clips. Sham operated animals received the same treatment; although the dura above the bulbs was punctured the actual bulbs were left intact. The animals were given 14 days to recover following surgery prior to drug administration and were handled daily to eliminate any aggressiveness that may otherwise arise (Leonard and Tuite, 1981).

2.3. Drug treatment

Olfactory bulbectomised and sham operated animals were each assigned to 12 treatment groups ($n = 7–14$) to which paroxetine (2.5 mg/kg i.p.), (SmithKline Beecham, Harlow, UK) dissolved in dimethyl sulphoxide (Sigma, Sydney) or vehicle (dimethyl sulphoxide), were administered for either 3, 7 or 14 days. Animals were pre-treated with either pindolol (2 mg/kg bid s.c.), (RBI, Natick, MA, USA) or vehicle (dimethyl sulphoxide).

2.4. Open field

The 'open field' test was conducted on bulbectomised rats and their sham operated controls on the morning following the final day of drug administration, day 15 of the study. Each rat was placed singly into the centre of the 'open field' apparatus (Gray and Lalljee, 1974). This apparatus consisted of a circular base, 90 cm in diameter which was divided into three circular sectors. The first consists of a circle in the middle of apparatus 10 cm in diameter, the next consists of a circle subdivided into eight segments from the inner circle out. This has a diameter of 50 cm and the final sector consists of the space between the middle circle and the outer wall and is subdivided into 16 equivalent sectors. All areas are marked by faint black lines. The wall surrounding the base is bright in colour (75 cm in height). Illumination was provided by a 60-W bulb, positioned 90 cm above the floor of the apparatus. All measurements were carried out in a darkened room. The number of segments crossed by each rat over a 3-min period was recorded.

2.5. Effect of 8-OH-DPAT on rectal temperature

The effect of 8-OH-DPAT (RBI Natick) on rectal temperature of each rat was determined on the last day of drug administration 6 h following that days initial dose. Each rat was challenged with an injection of 8-OH-DPAT (0.15 mg/kg, s.c.). Basal body temperature had been taken by

inserting a digital thermometer 4 cm into the rectum immediately prior to and 30 and 60 min following the challenge.

2.6. Statistical analysis

Initially a two-way analysis of variance was performed on the temperature data. If any statistically significant changes were found the data was further analysed using post-hoc Tukey's honest significantly difference test. All results were considered significant at $P < 0.05$. The ambulation data was analysed using a Kruskal–Wallis analysis of variance. If any statistically significant differences were found, the data was analysed using a Mann–Whitney *U*-test, $P < 0.05$.

3. Results

3.1. 'Open field' test

There was a significant increase in open field ambulation in the olfactory bulbectomised control group when compared to its corresponding sham operated animal following 3 days, ($H = 21.78$, $df = 7$, $P = 0.0028$), 7 days, ($H = 28.84$, $df = 7$, $P = 0.0002$) and 14 days of treatment ($H = 38.49$, $df = 7$, $P < 0.0001$). Paroxetine alone significantly attenuated this increase following 14 days of treatment only, whereas all other treatment regimes at all time points failed to alter this hyperactivity (Fig. 1).

3.2. 8-OH-DPAT challenge

There was a significant drop in temperature recorded in control animals 30 min after the 8-OH-DPAT challenge following 3, 7 and 14 days. There was no significant lesion effect on the degree of hypothermia observed at any time point. Following 3 days of treatment, there was a significant effect of drug on 8-OH-DPAT-induced hypothermia ($F_{(3,57)} = 6.17$, $P < 0.001$). Post-hoc analysis revealed a significant attenuation in the olfactory bulbectomised combination group, with a corresponding trend towards significance in the sham group. Neither of the other two groups had an altered hypothermic response to 8-OH-DPAT. Again, following 7 days of treatment, there was a significant effect of drug on 8-OH-DPAT-induced hypothermia ($F_{(3,59)} = 11.62$, $P < 0.0001$). Post-hoc analysis revealed a complete reversal of the hypothermic response in both the olfactory bulbectomised and sham operated combination groups. This attenuation was much more robust than that seen after 3 day-treatment. There was a slight trend towards attenuation of the hypothermia seen in both the paroxetine and the pindolol groups. Subsequent to 14 days of treatment, there was again a significant effect of drug on 8-OH-DPAT-induced hypothermia ($F_{(3,66)}$ 15.68, $P <$ 0.0001). Post-hoc analysis demonstrated that the hypothermic response was reversed in both the olfactory bulbectomised and sham operated paroxetine and combination groups. Pindolol alone failed to have any effect on 8-OH-DPAT-induced hypothermia in either olfactory bulbectomised or sham operated animals. A total of 60 min

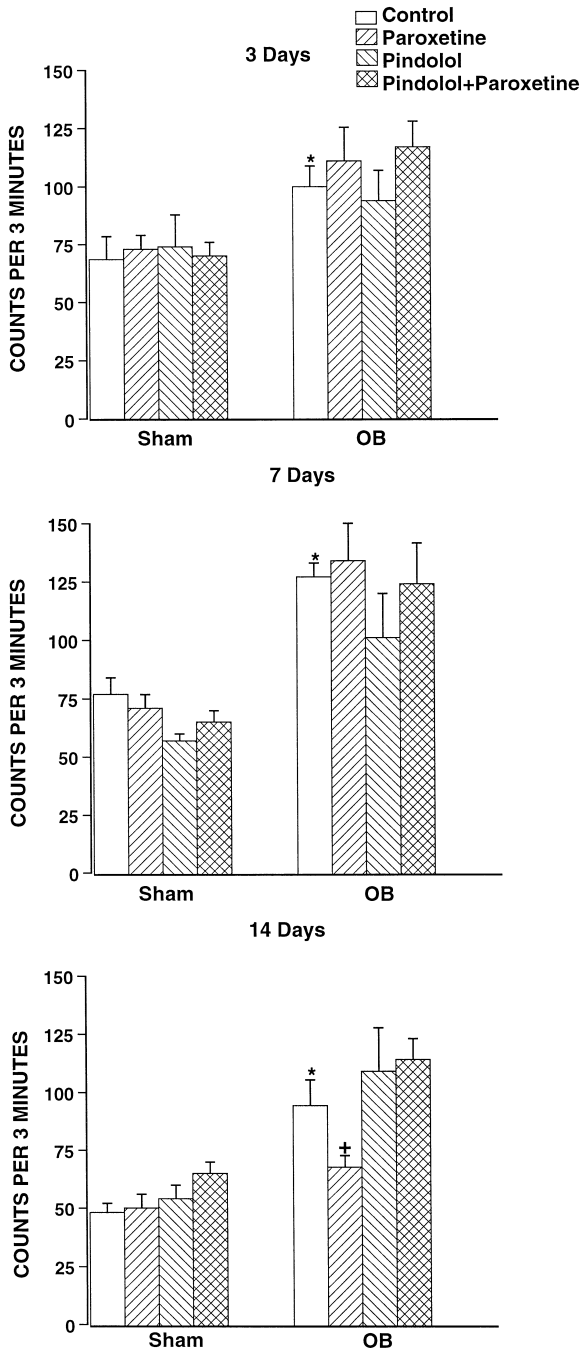

Fig. 1. The effects of pindolol and paroxetine in combination on olfactory bulbectomy-induced hyperactivity in the open field. Mean (+S.E.M.) ambulation scores were increased significantly in olfactory bulbectomised vehicle treated group at all time points. A 14-day treatment of paroxetine alone reduced this response significantly. No alteration in the ambulation scores was seen in other groups. $* P < 0.05$ vs. sham control; $+ P < 0.05$ vs. olfactory bulbectomised control (Kruskal–Wallis tests).

following the challenge, the hypothermic responses were returning to basal values in all affected groups (data not shown) (Fig. 2).

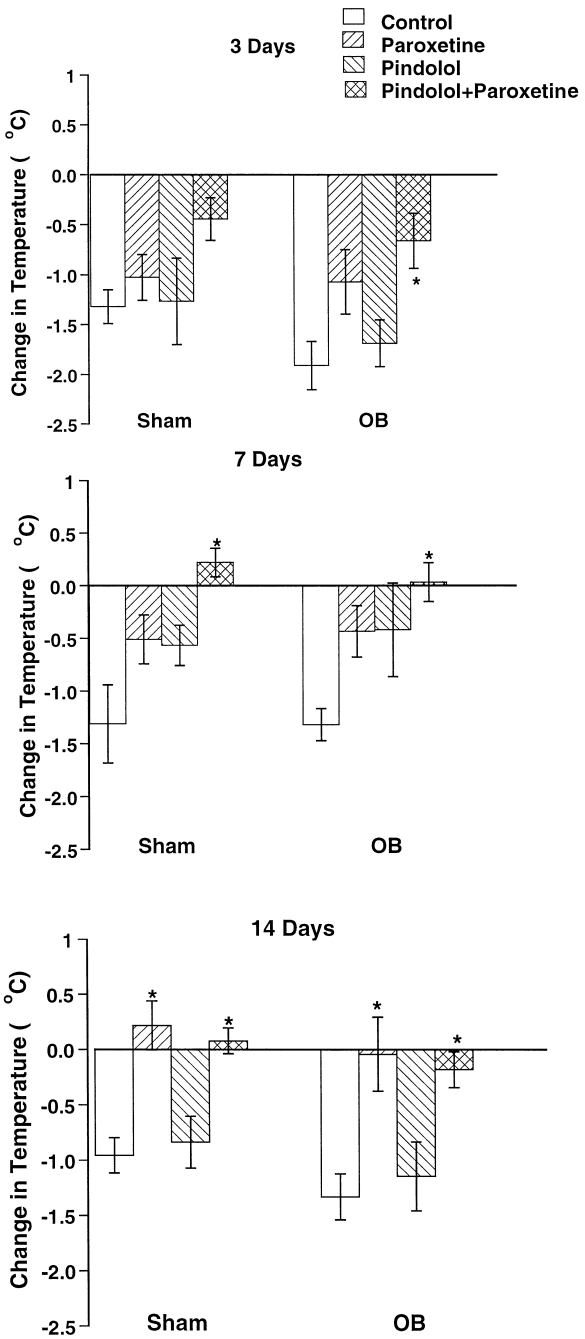

Fig. 2. The effects of pindolol and paroxetine treatment on 8-OH-DPAT-induced hypothermia in the olfactory bulbectomized rat. Following 3 days of treatment, a significant attenuation of 8-OH-DPAT induced hypothermia was seen in paroxetine + pindolol olfactory bulbectomised group only. By day 7, the hypothermic response was fully reversed in both olfactory bulbectomised and sham animals treated with the combination. Following 14 days of treatment, both the combination and paroxetine treated animals significantly reversed the hypothermic response whereas pindolol alone had no effect. Mean (+S.E.M.) is shown. $* P < 0.05$ vs. relevant control (Tukey's HSD tests).

4. Discussion

This attenuation of the olfactory bulbectomy-induced hyperactivity by paroxetine after 14 days of treatment is consistent with previous studies with the model (McGrath, 1996). However, the inability of the combined pindolol/paroxetine group to reverse the bulbectomy-induced hyperactivity at any time point is counterintuitive. It suggests that the olfactory bulbectomy model is not sensitive to the detection of an earlier onset of antidepressant action due to the paroxetine and pindolol combination. However, it poses intriguing questions into the mechanism of action of antidepressants in this model.

The olfactory bulbectomised rat model is sensitive to the actions of most clinically effective antidepressants after chronic administration only (see Kelly et al., 1997). In addition, it has many neurochemical, endocrine and immunological changes that correspond with those seen in clinical depression (Jesberger and Richardson, 1988; Song and Leonard, 1995). However, the exact biochemical basis of the effects of antidepressants in this model are far from understood (McGrath, 1996). Clearly, the present data suggests that the action of pindolol has the ability to antagonise the effect of paroxetine on the hyperactivity of the bulbectomised rats in the open-field apparatus. This is the first time any such reversal has been demonstrated. Further work is necessary to elucidate the neurochemical basis of this effect and whether it may be due to the β-adrenoceptor blockade actions of pindolol. It is unlikely to be due to its 5-HT_{1A} receptor antagonistic properties as the full antagonist WAY-100635 (*N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride) does not interfere with the open field effects of paroxetine in this model (Cryan et al., unpublished observations). Interestingly, a recent study has shown that the some of the olfactory bulbectomy-induced behavioural responses in the open field were reversed by another antidepressant; sertraline with and without pindolol (Thielen and Frazer, 1996). However, their study failed to demonstrate any olfactory bulbectomy-induced increase in total activity in the open field and therefore it is difficult to interpret these findings.

In addition, an alteration to the 5-HT_{1A} receptor following combined pindolol and paroxetine treatment in both sham and olfactory bulbectomised operated animals has been demonstrated. This adaptational change, as manifested by the attenuation of 8-OH-DPAT-induced hypothermia was faster with the combined treatment than with paroxetine alone. This further emphasises the role of this receptor subtype in the mechanism of action of antidepressant drugs, as well as supporting the concept of using the 5-HT_{1A} receptor as a target for development of faster acting antidepressants.

Whether this hypothermic response is a pre- or post-synaptic 5-HT_{1A} receptor effect remains controversial (see De Vry, 1995), and complicates the interpretation of the

present findings. Chronic paroxetine treatment has previously been suggested to cause a desensitisation of both pre- and postsynaptic 5-HT_{1A} receptors (Sargent et al., 1997), while pindolol may be a more specific antagonist at the presynaptic receptor (Meltzer and Maes, 1996; Artigas et al., 1996) although it has some postsynaptic 5-HT_{1A} receptor properties (Aulakh et al., 1988). If it is assumed that pindolol selectively blocks presynaptic (somatodendritic) 5-HT_{1A} receptors while the concurrent reuptake blockade by paroxetine causes an increase in synaptic 5-HT, it is hypothesised that the enhanced inter-synaptic concentrations of 5-HT may lead to a rapid postsynaptic 5-HT_{1A} receptor desensitisation/downregulation and hence a blunting of the hypothermic response.

While the mechanism of action of the pindolol–paroxetine combination may be disputed, we have shown that this paradigm is sensitive to potential faster acting antidepressant combinations, and is able to detect 5-HT_{1A} receptor mediated effects as early as 3 days after commencing treatment. The changes in 5-HT_{1A} receptor sensitivity do not seem to be related to the direct action of paroxetine on the 5-HT transporter in the olfactory bulbectomy model. This is consistent with the lack of a lesion effect in the hypothermic response between olfactory bulbectomised and sham operated animals in this and previous studies (Cryan et al., 1997). The attenuation of 8-OH-DPAT-induced hypothermia is a model of 5-HT_{1A} receptor function rather than one of antidepressant action, and there have been inconsistent effects seen with some antidepressants, (Lund et al., 1992; Wozniak et al., 1988; McGrath, 1996; Cryan et al., unpublished observations).

The clinical findings with pindolol, although suggesting a faster acting antidepressant response, have yet to demonstrate this unequivocally (Berman et al., 1997). It therefore would be premature to rule out the olfactory bulbectomy model completely as a method of detecting faster acting antidepressants until a positive control is available. However, the quest remains to find or refine an animal model sensitive to the early onset of effects predictive of clinical antidepressant activity.

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