Br. J. Pharmacol. (1990), **99**, 221–222 **SHORT COMMUNICATION**

Effects of a selective 5-HT_2 agonist, DOI, on 5-HT neuronal firing in the dorsal raphe nucleus and 5-HT release and metabolism in the frontal cortex

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Systemic administration of the 5-HT_2 agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) (50 and 100 $\mu g\,kg^{-1}$, i.v.) inhibited dorsal raphe neuronal firing. DOI (100 $\mu g\,kg^{-1}$, i.v.) also produced a decrease in extracellular 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in the frontal cortex measured by microdialysis. However, local administration of DOI into the frontal cortex produced no change in extracellular 5-HT and 5-HIAA at any dose given (1, 10 and 100 ng). The results demonstrate that DOI is a potent inhibitor of 5-HT neuronal firing and terminal release and that the effects on release are not mediated by an action within the terminal region. The site of action and the receptor involved in the inhibition remains to be determined.

Introduction 5-Hydroxytryptamine (5-HT) receptors can be divided into number of types of which the 5-HT_{1A} and 5-HT_2 subclasses are most thoroughly characterized (Glennon, 1987). The 5-HT_{1A} selective agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OHDPAT) (Hjorth *et al.*, 1982) is a potent inhibitor of dorsal raphe nucleus (DRN) neuronal firing via action on autoreceptors located on the cell bodies and/or dendrites (but not presynaptic terminals) of ascending 5-HT neurones in the DRN (Verge *et al.*, 1985). Systemic administration of the 5-HT_2 agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) (Glennon, 1987) has been found to inhibit DRN neuronal firing (Garratt & Marsden, 1989). Changes in DRN neuronal function normally lead to corresponding changes in 5-HT release and metabolism in the regions containing 5-HT terminals (Marsden *et al.*, 1989).

This study investigated the effect of DOI on DRN 5-HT neuronal firing and release and metabolism in the frontal cortex to determine whether this 5-HT_2 agonist altered 5-HT release in the frontal cortex *in vivo* and whether effects observed were due to a direct effect of DOI in the frontal cortex or secondary to changes in DRN neuronal firing.

Methods In all experiments male Wistar rats (250–300 g) were housed in groups of five under a 12 h light-dark cycle and allowed food and water *ad libitum*. They were anaesthetized with halothane (1.5–2%)/O_2/N_2O, the jugular vein was cannulated to allow intravenous administration of drugs.

Electrophysiology Single barrelled glass electrodes with an *in vitro* impedance of 4–6 MΩ were used. These were filled with 2 M NaCl containing 2% pontamine sky blue. The animal was placed in a stereotaxic frame and a hole was drilled 7.8 mm posterior of bregma on the midline. The dorsal raphe nucleus is located 5.0–6.5 mm below the dura. 5-HT cells were physiologically identified by the criteria of Aghajanian *et al.* (1968), and pharmacologically by the intravenous administration of 8-OHDPAT which temporarily reduces DRN 5-HT cell firing (Figure 1a). DOI (50 or 100 $\mu g\,kg^{-1}$, i.v.) or control injection was then administered. After a recording, the location of the cell was marked with a spot of dye permitting the confirmation of the electrode position histologically.

Dialysis Dialysis probes were constructed and perfused with artificial CSF (pH 7.4) at a rate of 1 $\mu l\,min^{-1}$. The dialysis probe was implanted into the frontal cortex (rostal-caudal +3.6 mm from bregma, saggital ±1.5 mm from bregma, vertical 2.5 mm from the dura). For local injections a guide cannula was implanted alongside the dialysis probe. From 2 h after implantation, dialysis samples were collected every 20 min.

H.p.l.c. assay of 5-HT and 5-HIAA 5-HT and 5-HIAA were separated by ion-pair, reverse phase chromatography, by use of column packed with 3 μm Hypersil. Mobile phase was pumped through the column at a flow rate of 0.25 $ml\,min^{-1}$. The amines eluted from the column were measured with a dual glassy carbon working electrode maintained at a potential of +0.85 V. Basal levels were measured for 80 min after which one group of rats received either systemic DOI (100 $\mu g\,kg^{-1}$, i.v.) or saline (i.v.) while the other group was given either DOI (1, 10 and 100 ng in 0.5 μl CSF) or CSF (0.5 μl) into the frontal cortex and dialysis samples collected for a further 180 min.

Results DOI (50 $\mu g\,kg^{-1}$, i.v.) produced a 60% inhibition of the firing rate, the higher dose (100 $\mu g\,kg^{-1}$, i.v.) caused complete cessation of DRN 5-HT neuronal firing for over an hour (Figure 1a). In the frontal cortex the higher dose of DOI produced a decrease in both extracellular 5-HT (44 ± 10.2% of saline control) and 5-HIAA (32 ± 7.4% of saline control) (Figure 1b). DOI (1, 10, and 100 ng) administered locally in the frontal cortex produced no change in either extracellular 5-HT or 5-HIAA at any dose given (Figure 1c).

Discussion The results demonstrate that the 5-HT_2 agonist DOI is a potent inhibitor of DRN 5-HT neuronal firing. Furthermore, the effects of DOI appear to be dose-related. Although DOI has produced a similar effect to 8-OHDPAT it is unlikely that the inhibition seen with DOI is due to an action at the 5-HT_{1A} site since the affinity of DOI for this site is very low (Glennon, 1987) and it would require large doses of DOI for any binding to 5-HT_{1A} sites to occur. Autoradiographic studies have shown a high density of postsynaptic 5-HT_2 sites in the cortex (Hoyer *et al.*, 1986) and more recent studies using [^{125}I]-DOI revealed highest binding in the

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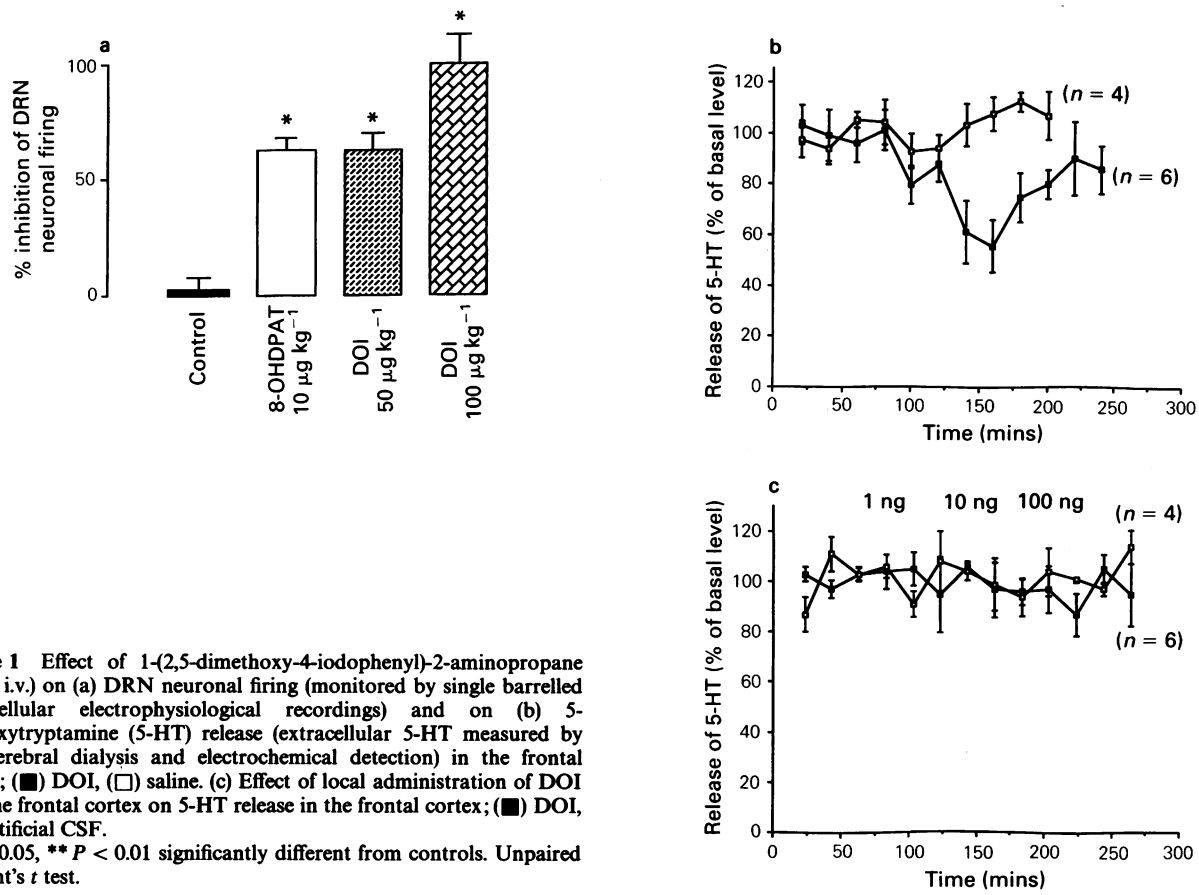

Figure 1 Effect of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI, i.v.) on (a) DRN neuronal firing (monitored by single barrelled extracellular electrophysiological recordings) and on (b) 5-hydroxytryptamine (5-HT) release (extracellular 5-HT measured by intracerebral dialysis and electrochemical detection) in the frontal cortex; (■) DOI, (□) saline. (c) Effect of local administration of DOI into the frontal cortex on 5-HT release in the frontal cortex; (■) DOI, (□) artificial CSF.
* $P < 0.05$, ** $P < 0.01$ significantly different from controls. Unpaired Student's t test.

choroid plexus with high binding in the frontoparietal cortex and nucleus accumbens (McKenna *et al.*, 1989). As systemic but not local injection (frontal cortex) of DOI produced a significant decrease in both 5-HT release and metabolism in the frontal cortex it would appear that the decrease in release is not a result of a direct action of DOI on these [^{125}I]-DOI binding sites within the terminal region and indirectly supports evidence that the 5-HT$_2$ binding sites in the frontal cortex are located postsynaptically.

It is possible that the effects of DOI described in this paper may not be due to an action on the classical 5-HT$_2$ site since initial binding data suggested that [^{125}I]-DOI labelled a high-affinity state of the 5-HT$_2$ recognition site (Glennon *et al.*, 1988). McKenna & Peroutka (1989) have shown that structural congeners of R-(−)-DOI display a higher affinity for the [^{125}I]-R-(−)-DOI binding site than the [^{3}H]-ketanserin labelled binding site, which may suggest that there are two subtypes of the 5-HT$_2$ site.

The method(s) of action and receptor type(s) through which DOI produces inhibition of DRN 5-HT neuronal firing and decreases 5-HT release and metabolism warrant further investigation.

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