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Determination of the role of noradrenergic and 5-hydroxytryptaminergic neurones in postsynaptic α_2-adrenoceptor desensitization by desipramine and ECS

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1 Experiments were conducted to determine the respective roles which noradrenergic and 5-hydroxytryptaminergic neurones play in the down-regulation of postsynaptic α_2-adrenoceptors by desipramine and electroconvulsive shock (ECS). The functional status of these receptors was monitored by use of clonidine-induced mydriasis in conscious mice.

2 Mydriasis to clonidine (0.1 mg kg^{-1}, i.p.) was markedly attenuated by administration of either desipramine (10 mg kg^{-1}, i.p.) for 14 days or ECS (200 V, 2 s) given five times over ten days confirming our previous observations.

3 The neurotoxin, DSP-4 (100 mg kg^{-1}, i.p. × 2), reduced brain noradrenaline levels by 64% and abolished the mydriasis induced by the noradrenaline releasing agent and reuptake inhibitor, methamphetamine, without significantly altering the response to clonidine, confirming our earlier results. This lesion prevented the attenuation of clonidine mydriasis by repeated administration of desipramine, but not ECS.

4 Lesioning of central 5-hydroxytryptaminergic neurones with 5,7-dihydroxytryptamine (75 μg, i.c.v.) had no influence on the reduction in clonidine mydriasis produced by repeated administration of either desipramine or ECS.

5 Since noradrenergic neurones are essential for the desensitization of postsynaptic α_2-adrenoceptors by desipramine, it indicates that this effect is probably the result of increased synaptic noradrenaline levels. This mechanism is not responsible for the change induced by ECS because this adaptation is independent of an intact noradrenergic input. 5-HT-containing neurones do not play a permissive role in the down-regulation of postsynaptic α_2-adrenoceptors by either antidepressant treatment.

Keywords: Clonidine-induced mydriasis; α_2-adrenoceptors; postsynaptic α_2-adrenoceptors; brain; antidepressants; antidepressant effects; desipramine; electroconvulsive shock

Introduction

It is now accepted that prolonged administration of many antidepressant drugs produces changes in the function of the monoamine neurotransmitters, noradrenaline and 5-hydroxytryptamine (5-HT). The most familiar of these adaptive effects are probably the down-regulation of β-adrenoceptors (Schultz, 1976; Vetulani *et al.*, 1976a,b) and 5-HT$_2$ receptors (Peroutka & Snyder, 1980; Blackshear *et al.*, 1982; Goodwin *et al.*, 1984; Metz & Heal, 1986). However, the concept that down-regulation results exclusively from an increase in the synaptic concentrations of these neurotransmitters is too simplistic to explain the actions of all antidepressant treatments. For example, the down-regulation of β-adrenoceptors by desipramine is dependent upon intact noradrenergic function (Vetulani *et al.*, 1976a; Dooley *et al.*, 1983; Hall *et al.*, 1984), whereas electroconvulsive shock (ECS) decreases these receptors even after destruction of noradrenergic neurones (Vetulani & Sulser, 1975; Kellar *et al.*, 1981; Dooley *et al.*, 1987). In addition, there is also evidence to show that antidepressant-induced adrenoceptor desensitization involves a functional interplay between central noradrenergic and 5-hydroxytryptaminergic neurones. Thus, inhibition of 5-HT synthesis or denervation with 5,7-dihydroxytryptamine (5,7-DHT) prevents either the down-regulation of β-adrenoceptors by antidepressants (Brunello *et al.*, 1982; 1985; Nimgaonkar *et al.*, 1985) or, possibly, the maintenance of this effect (Asakura *et al.*, 1987).

Recently, we have demonstrated that clonidine-induced mydriasis measured in conscious C57/B1/6 mice is specifically mediated by postsynaptic α_2-adrenoceptors in the brain (Heal *et al.*, 1989a,b). Moreover, this functional response is attenuated after repeated administration of either antidepressants which increase central noradrenergic function or repeated ECS (Heal *et al.*, 1991). In experiments analogous to those previously conducted on β-adrenoceptors, we have now determined the roles which noradrenergic and 5-hydroxytryptaminergic neurones play in the attenuation of postsynaptic α_2-adrenoceptor function by administration of desipramine and ECS.

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Methods

Animals

Adult male C57/B1/601a mice (Olac) weighing 25–30 g were used. They were housed in groups of six to ten on a 12 h light/dark cycle (commencing 06 h 00 min) at a temperature of 21°C and 55% humidity. Mice were allowed free access to food and water.

Lesioning of noradrenaline- and 5-HT-containing neurones in the brain

Destruction of brain noradrenergic neurones was carried out by use of the selective neurotoxin, DSP-4. Mice were pretreated with zimeldine (5 mg kg^{-1}, i.p.) to protect central 5-HT-containing neurones and DSP-4 (100 mg kg^{-1}, i.p.) was administered 30 min later. This procedure was repeated seven days later. After a further three days, verification of extensive noradrenergic denervation was initially obtained by measurement of methamphetamine (0.5 mg kg^{-1}, i.p.)-induced

mydriasis in all DSP-4 lesioned mice as this response is abolished in mice after depletion of brain noradrenaline levels by approximately 75% (Heal et al., 1989b). To demonstrate that no shift in the basal mydriasis response had occurred after DSP-4 lesioning, groups of mice were randomly selected from the methamphetamine non-responders and sham-lesioned controls and their response to clonidine (0.1 mg kg^{-1}, i.p.) was determined.

5-Hydroxytryptaminergic neurones were lesioned by use of the selective neurotoxin, 5,7-DHT. Mice were initially pretreated with desipramine (25 mg kg^{-1}, i.p.) to protect noradrenaline-containing neurones and 15 min later the animals were anaesthetized with hexobarbitone (50 mg kg^{-1}, i.p.). 5,7-DHT (75 μg) dissolved in 4 μl ice-cold saline containing 0.4 mg ml^{-1} ascorbic acid was injected intracerebroventricularly (i.c.v.) with the stereotaxic apparatus described by Heal (1984). Sham-lesioned mice were injected with vehicle (4 μl, i.c.v.). Mice were then housed individually and left for 14 days to recover. Verification of substantial 5-HT depletion was initially performed by measurement of 5-methoxy-N,N-dimethyltryptamine-induced head-twitches and finally by determination of neurotransmitter concentrations by high performance liquid chromatography (h.p.l.c.) with electrochemical detection. Full details of these procedures, together with the reduction in 5-HT levels, are given in Heal et al. (1990). To demonstrate that destruction of 5-HT-containing neurones had not affected α_2-adrenoceptor-mediated mydriasis, groups of mice were randomly selected from the acceptable 5,7-DHT-lesioned and sham-lesioned animals and these were tested with clonidine (0.1 mg kg^{-1}, i.p.).

Administration of desipramine and electroconvulsive shock

Mice injected with DSP-4 (100 mg kg^{-1}, i.p. × 2) or saline were each divided into two subgroups. They were injected with desipramine (10 mg kg^{-1}, i.p.) or saline (0.25 ml, i.p.) once daily for 14 days. In a second experiment, subgroups of DSP-4-treated and sham-lesioned mice were anaesthetized with a constant flow air-halothane mixture and given an ECS (200 V, 2 s) five times over 10 days (Mon, Wed, Fri, Mon, Wed). Controls were anaesthetized.

Groups of mice injected with 5,7-DHT (75 μg, i.c.v.) or saline-ascorbate (4 μl, i.c.v.) were subdivided and treated with desipramine or ECS according to the protocols described above.

Clonidine-induced mydriasis was measured 24 h after the final desipramine or ECS treatment.

Measurement of α_2-adrenoceptor-mediated mydriasis

A Wild M1 binocular microscope with graticule scale in one eyepiece was used to measure pupil diameter. Illumination of the microscope was provided by a Swift light box with the voltage set at 6 V (light intensity 2500 lux). The procedure was carried out in an artificially lit room (light intensity 650 lux). The mouse was held beneath the microscope and its pupil diameter was read off in eyepiece units. This figure was later converted to millimetres. The mouse was injected with either clonidine (0.1 mg kg^{-1}, i.p.) or methamphetamine (0.5 mg kg^{-1}, i.p.) and its pupil diameter was measured again 10 min later. A dose of 0.1 mg kg^{-1} of clonidine was chosen because it allowed for the detection of either enhancement or inhibition of this response (Heal et al., 1989a).

Measurement of brain monoamine concentrations by h.p.l.c. with electrochemical detection

Measurement of brain monoamines (noradrenaline, 5-HT and dopamine) was performed by h.p.l.c. with electrochemical detection to confirm the selectivity and extent of the DSP-4 and 5,7-DHT lesioning procedures. All lesioned and sham-lesioned mice used in the desipramine and ECS experiments were killed 24 h after the measurement of clonidine-induced mydriasis. Brains were homogenized in five volumes (w/v) of 0.1 M perchloric acid containing 0.4 mM sodium metabisulphite (antioxidant) and 0.8 μM isoprenaline (internal standard) in a Polytron PT 10–35 disruptor (setting 5–6). After centrifugation at 1100 g for 15 min at 4°C and 15000 g for 5 min at 4°C, 30 μl of the resulting supernatant was injected onto the h.p.l.c. system for the determination of monoamine concentrations. This comprised a DuPont 870 pump (flow rate 1 ml min^{-1}) connected via a WISP 712 refrigerated autoinjector to a 5 μm Hypersil ODS 1 reversed-phase analytical column (length 250 × 4.6 mm i.d.) maintained at 45°C and protected by a Brownlee Aquapore RP-300 precolumn (length 30 × 4.6 mm i.d.). The h.p.l.c. mobile phase was 0.1 M sodium dihydrogen orthophosphate-orthophosphoric acid buffer pH 3.2 containing 16% v/v methanol, 2.8 mM 1-octane sulphonic acid sodium salt and 0.7 mM EDTA. Noradrenaline, 5-HT and dopamine were detected by use of a BAS LC-4A amperometric detector with a TL-5 flow-cell set at a potential of +0.75 V versus an Ag/AgCl reference electrode.

Drugs and reagents

Drugs were obtained from the following sources: clonidine HCl, desipramine HCl, 5,7-dihydroxytryptamine creatinine sulphate (5,7-DHT), hexobarbitone, methamphetamine HCl (Sigma, Poole); (−)-ascorbic acid (FSA, Loughborough); halothane (May and Baker, Dagenham); N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4; Semat, St. Albans). Drugs for intraperitoneal injection were dissolved in 0.9% (w/v) sodium chloride solution (saline) and were administered in weight-related doses (0.1 ml 10 g^{-1} body weight).

Reagents for h.p.l.c. analysis were of the highest purity available and were obtained from the following sources: dopamine HCl, 5-hydroxytryptamine creatinine sulphate (5-HT), isoprenaline HCl, noradrenaline HCl, sodium metabisulphite (Sigma, Poole); perchloric acid, orthophosphoric acid (BDH, Poole); 1-octane sulphonic acid sodium salt, sodium dihydrogen orthophosphate (FSA, Loughborough); ethylenediaminetetraacetic acid disodium salt (EDTA) (Aldrich, Gillingham); methanol (Rathburn Chemicals, Walkerburn). All water was distilled and deionised before used.

Statistics

Results were statistically evaluated by Student's unpaired t test.

Results

Effects of noradrenergic denervation

Effects of DSP-4 lesioning on methamphetamine- and clonidine-induced mydriasis DSP-4 (100 mg kg^{-1}, i.p. × 2) was administered to mice as outlined in Methods. Mice were tested for their mydriatic response to methamphetamine (0.5 mg kg^{-1}, i.p.) three days after the second DSP-4 treatment. DSP-4 lesioning abolished the methamphetamine-induced response when compared with sham-lesioned controls (Figure 1a). A subgroup of these mice was tested with clonidine (0.1 mg kg^{-1}, i.p.) 24 h later. Although DSP-4 lesioning produced a small enhancement of this response, the potentiation was not significant (Figure 1b).

Influence of DSP-4 lesioning on the attenuation of clonidine-induced mydriasis by desipramine or ECS Groups of mice injected with DSP-4 (100 mg kg^{-1}, i.p. × 2) or saline were injected with desipramine (10 mg kg^{-1}, i.p.) once daily for 14 days, while controls received saline (0.25 ml, i.p.). When the

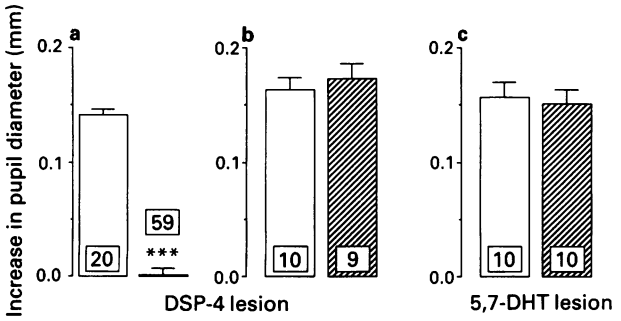

Figure 1 The effects of DSP-4 and 5,7-dihydroxytryptamine (5,7-DHT) lesioning on α_2-adrenoceptor-mediated mydriasis responses. (a and b) Groups of mice were injected with DSP-4 (100 mg kg^{-1}, i.p. × 2; hatched columns) or saline (0.25 ml, i.p. × 2; open columns). (c) Groups of mice were injected with 5,7-DHT (75 μg, i.c.v; hatched columns) or saline (4 μl, i.c.v.; open columns). All lesioning procedures are fully described in Methods. The results are the increase in pupil diameter (mm) (s.e.mean shown by vertical bars) measured 10 min after (a) methamphetamine (0.5 mg kg^{-1}, i.p.) or (b and c) clonidine (0.1 mg kg^{-1}, i.p.). The number of mice tested is shown in each column. Significantly different from appropriate control group: *** $P < 0.001$.

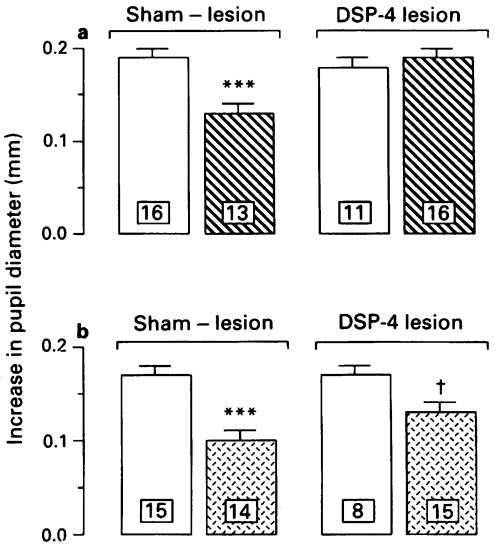

Figure 2 The influence of noradrenergic denervation on the attenuation of clonidine-induced mydriasis by desipramine or electroconvulsive shock (ECS). Groups of DSP-4 (100 mg kg^{-1}, i.p. × 2)-treated and sham-lesioned mice were: (a) injected with desipramine (10 mg kg^{-1}, i.p.; hatched columns) or saline (0.25 ml, i.p.; open columns) once daily for 14 days or (b) given an ECS (200 V, 2 s; stippled columns) or halothane (open columns) five times over 10 days. Twenty-four hours after the final treatment, the mydriasis induced by clonidine (0.1 mg kg^{-1}, i.p.) was measured. The results are increase in pupil diameter (mm) (s.e.mean shown by vertical bars) measured 10 min after clonidine injection with the number of mice tested shown in each column. Significantly different from appropriate control group: † $P < 0.02$; *** $P < 0.001$.

mydriasis response to clonidine (0.1 mg kg^{-1}, i.p.) was measured 24 h after the final dose of desipramine, DSP-4 lesioning was found to prevent the attenuation of clonidine-induced mydriasis by repeated desipramine treatment (Figure 2a).

When groups of DSP-4-treated and sham-lesioned mice were given either an ECS (200 V, 2 s) five times over 10 days or halothane, DSP-4 treatment did not influence the reduction in clonidine-induced mydriasis induced by repeated ECS (Figure 2b).

Effects of DSP-4 treatment on brain monoamine concentrations Mice were killed 24 h after the determination of clonidine-induced mydriasis and brain concentrations of noradrenaline, 5-HT and dopamine were measured by h.p.l.c. with electrochemical detection as detailed in Methods. DSP-4 (100 mg kg^{-1}, i.p. × 2) treatment markedly reduced brain noradrenaline concentrations with only small alterations in 5-HT and dopamine levels (Table 1). The extent of noradrenaline depletion produced by DSP-4 was almost identical in both the desipramine and ECS experiments (Table 1).

Effects of 5-hydroxytryptaminergic denervation

Effect of 5,7-DHT lesioning on clonidine-induced mydriasis Fifteen days after 5,7-DHT (75 μg, i.c.v.) injection, a subgroup of the mice was tested with clonidine (0.1 mg kg^{-1}, i.p.). 5,7-DHT lesioning (for extent of 5-HT lesion see Heal *et al.*, 1990) had no significant effect on the mydriasis response when compared with sham-lesioned controls (Figure 1c).

Influence of 5,7-DHT lesioning on the attenuation of clonidine-induced mydriasis by desipramine or ECS Groups of 5,7-DHT (75 μg, i.c.v.)-treated and sham-lesioned mice were injected with desipramine (10 mg kg^{-1}, i.p.) once daily for 14 days, while controls received saline (0.25 ml, i.p.). The attenuation of clonidine (0.1 mg kg^{-1}, i.p.)-induced mydriasis by repeated desipramine treatment was unaffected by 5,7-DHT lesioning (extent of lesioning given in Heal *et al.*, 1990), when measured 24 h after the final dose of this antidepressant (Figure 3a).

When groups of 5,7-DHT-lesioned and sham-lesioned mice were given either an ECS (200 V, 2 s) five times over 10 days or halothane, 5,7-DHT treatment (extent of lesioning given in Heal *et al.*, 1990) did not affect the attenuation of clonidine-induced mydriasis by repeated ECS (Figure 3b).

Table 1 The effects of DSP-4 lesioning on mouse brain monoamine concentrations

	Brain concentrations		
	Noradrenaline	*5-HT*	*Dopamine*
Desipramine experiment			
Sham-lesioned	707 ± 6 (29)	842 ± 8	1374 ± 9
DSP-4 (100 mg kg^{-1} × 2)	258 ± 3 (27)***	903 ± 11***	1284 ± 14***
% depletion	64	−7	7
ECS experiment			
Sham-lesioned	690 ± 6 (29)	792 ± 12	1503 ± 22
DSP-4 (100 mg kg^{-1} × 2)	235 ± 3 (23)***	754 ± 18*	1239 ± 15***
% depletion	66	5	18

Mice were lesioned with DSP-4 (100 mg kg^{-1}, i.p. × 2) as detailed in Methods. The results are brain monoamine concentration (ng g^{-1} tissue wet wt) ± s.e.mean. The numbers of mice used are shown in parentheses in the left hand column. Significantly different from appropriate sham-lesioned control group: * $P < 0.05$; *** $P < 0.001$.

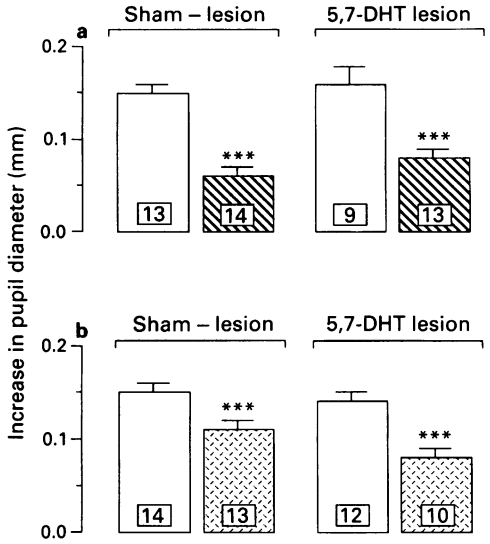

Figure 3 The influence of 5-hydroxytryptaminergic denervation on the attenuation of clonidine-induced mydriasis by desipramine or electroconvulsive shock (ECS). Groups of 5,7-dihydroxytryptamine (5,7-DHT, 75 μg, i.c.v.)-treated and sham-lesioned mice were: (a) injected with desipramine (10 mg kg^{-1}, i.p.; hatched columns) or saline (0.25 ml, i.p.; open columns) once daily for 14 days or (b) given an ECS (200 V, 2 s; stippled columns) or halothane (open columns) five times over 10 days. Twenty-four hours after the final treatment, the mydriasis induced by clonidine (0.1 mg kg^{-1}, i.p.) was measured. The results are increase in pupil diameter (mm) (with s.e.mean shown by vertical bars) measured 10 min after clonidine injection with the number of mice tested shown in each column. Significantly different from appropriate control group: *** $P < 0.001$.

Discussion

In this study, repeated administration of desipramine or ECS attenuated postsynaptic α_2-adrenoceptor function as indicated by clonidine-induced mydriasis and both findings are in agreement with earlier observations obtained by use of the mydriasis response in mice (Heal *et al.*, 1991) or rats (Menargues *et al.*, 1990). Since ligand-receptor binding techniques also predominantly monitor changes in the postsynaptic α_2-adrenoceptor population (U'Prichard *et al.*, 1980; Dooley *et al.*, 1983; Payvandi *et al.*, 1990), there is some value in comparing findings from these studies with those obtained with the clonidine-induced mydriasis model, although it should be noted that the anatomical sites involved are different, i.e. mydriasis results from activation of α_2-adrenoceptors in the Edinger-Westphal nucleus (Sharpe & Pickworth, 1981), whereas ligand-receptor binding is generally measured in the cortex. In agreement with the current findings, cortical α_2-adrenoceptors have been reported to be decreased by repeated treatment with desipramine or ECS (Smith *et al.*, 1981; Vetulani, 1982; Stanford & Nutt, 1982; Pilc & Vetulani, 1982; Heal *et al.*, 1987; 1989c). However, the former observation has been disputed by others (Johnson *et al.*, 1980; Asakura *et al.*, 1982; Sugrue, 1983; Stanford *et al.*, 1983).

DSP-4 lesioning was sufficiently complete to abolish the mydriasis evoked by methamphetamine, a noradrenaline releasing agent and reuptake inhibitor (Glowinski & Axelrod, 1965; Raiteri *et al.*, 1975), but did not suppress the response to the agonist, clonidine. However, in confirmation of our earlier findings (Heal *et al.*, 1989b), we observed that although there was a slight enhancement of clonidine-induced mydriasis, there was no evidence of marked denervation supersensitivity. Dooley *et al.* (1983) have also shown that DSP-4 lesioning did not increase α_2-adrenoceptors in all brain regions providing further evidence to indicate that certain populations of this receptor probably do not become supersensitive after denervation. If this hypothesis is correct, it provides the interesting phenomenon of a receptor that is easily down-regulated by increased noradrenergic function (Heal *et al.*, 1991) but is unresponsive to decreases in this neurotransmitter. Noradrenergic denervation prevented the decrease in clonidine-induced mydriasis produced by desipramine treatment, but did not impede the attenuation produced by ECS. These findings argue that the noradrenaline reuptake inhibitor, desipramine, probably causes adaption by increasing the synaptic concentrations of this neurotransmitter. By contrast, ECS almost certainly produces this effect by an indirect, but as yet, undefined mechanism and this hypothesis is supported by earlier studies showing that ECS has little effect on noradrenaline turnover or release (Nimgaonkar *et al.*, 1986; Green *et al.*, 1987). This finding of a differential necessity for an intact noradrenergic input in the attenuation of postsynaptic α_2-adrenoceptors by desipramine and ECS mirrors the previously reported requirements for the down-regulation of β-adrenoceptors by these two antidepressant treatments, i.e. noradrenergic neurones are essential for the down-regulation by desipramine, but not ECS (Vetulani & Sulser, 1975; Vetulani *et al.*, 1976a; Kellar *et al.*, 1981; Dooley *et al.*, 1983; 1987; Hall *et al.*, 1984). An alternative explanation is that it is the noradrenergic neurone (or possibly a cotransmitter contained within it), rather than noradrenaline *per se* which is essential for the down-regulation of α_2-adrenoceptors by desipramine. As an analogy, it has been reported that although 5-HT depletion with *p*-chlorophenylalanine does not influence desipramine-induced desensitization of β- or presynaptic α_2-adrenoceptors, 5,7-DHT lesioning abolished these effects, (Nimgaonkar *et al.*, 1986; Heal *et al.*, 1990) indicating that the 5-HT-containing neurone, but not 5-HT itself, is essential for the down-regulation process. However, this hypothesis is less plausible because studies which have examined the effects on mydriasis of monoamine depletion, as opposed to noradrenergic neuronal destruction, have clearly indicated that the latter do not have a functional role to play in the control of this response (Koss, 1979; Koss *et al.*, 1984).

In experiments to determine the influence which 5-HT function exerts on the antidepressant-induced attenuation of this postsynaptic α_2-adrenoceptor-mediated response, the indolamine-containing neurones were destroyed by intracerebroventricular injection of 5,7-DHT. By employing the method to determine sequentially clonidine-induced mydriasis and hypoactivity in the same animal (Heal *et al.*, 1989b), the mice used in the present study were those previously used to determine the influence of 5,7-DHT lesioning on the down-regulation of presynaptic α_2-adrenoceptors by desipramine and ECS in the study of Heal *et al.* (1990). Data reported by Heal *et al.* (1990) showed that this 5,7-DHT lesion produced a 77% reduction of brain 5-HT, with minimal effects on noradrenaline or dopamine levels, and produced a marked supersensitivity of 5-HT$_2$-mediated head-twitch behaviour. However, as shown in the present investigation, the denervation procedure did not alter clonidine-induced mydriasis or its reduction by repeated administration of either desipramine or ECS. The lack of a permissive role for 5-HT-containing neurones in the adaptation of postsynaptic α_2-adrenoceptors contrasts sharply with their involvement in the down-regulation of both presynaptic α_2- and β-adrenoceptors. Thus, clonidine-induced hypoactivity, which is an index of presynaptic α_2-adrenoceptor function in the brain (Heal *et al.*, 1989b), is attenuated by repeated administration of desipramine (Heal *et al.*, 1983) and ECS (Heal *et al.*, 1981). When 5-HT-containing neurones were destroyed by the 5,7-DHT lesioning protocol employed in this study, this procedure prevented the effects of the former, but not the latter, antidepressant treatment (Heal *et al.*, 1990). Similarly, 5,7-DHT lesioning has also been reported to inhibit the down-regulation of β-adrenoceptors by a variety of antidepressants, including desipramine and ECS (Brunello *et al.*, 1982; Nimgaonkar *et al.*, 1985). More recently, Asakura *et al.* (1987) have refined this hypothesis by arguing that 5-HT-containing neurones do not in fact play a permissive role in β-adrenoceptor down-regulation process, but

they are essential for the prolonged maintainance of this adaptive response.

Overall, the results presented demonstrate that intact noradrenergic neurones are essential for the attenuation of clonidine-induced mydriasis by desipramine, but not ECS. In addition, 5-HT-containing neurones do not play a permissive role in the antidepressant-induced adaptation of this postsynaptic α_2-adrenoceptor-mediated response.

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