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Stimulation of Corticosterone Secretion by the Selective 5-HT_{1A} Receptor Agonist 8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) in the Rat

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PRZEGALIŃSKI, E., B. BUDZISZEWSKA, A. WARCHOŁ-KANIA AND E. BŁASZCZYŃSKA. *Stimulation of corticosterone secretion by the selective 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) in the rat.* PHARMACOL BIOCHEM BEHAV **33**(2) 329–334, 1989.—The selective 5-HT_{1A} receptor agonist 8-OH-DPAT increased serum corticosterone concentration in rats in a dose-dependent manner. The synthetic corticoid dexamethasone lowered the serum corticosterone level and abolished its rise induced by 8-OH-DPAT. The corticosterone response to 8-OH-DPAT was also antagonized by spiperone, (±)- and (−)-pindolol and (±)-propranolol, all of which have been shown to have a high affinity for 5-HT_{1A} receptors, though in most cases no complete blockade was found. A partial antagonism of the response was also observed after flumazenil, a benzodiazepine antagonist. On the other hand, the 5-HT_{1B} receptor antagonist 21009, the 5-HT_2 receptor antagonists ketanserin and pirenperone, the 5-HT_3 receptor antagonist ICS 205-930, the α_2-adrenoceptor antagonists yohimbine and idazoxan, the β-adrenoceptor blocker with no affinity to 5-HT_1 receptors, atenolol, the dopaminergic antagonist pimozide, the histamine receptor blocker chloropyramine and the opiate receptor antagonist naloxone did not affect the hormonal response to 8-OH-DPAT. The 8-OH-DPAT-induced corticosterone secretion was not affected either in rats pretreated with p-chlorophenylalanine (PCPA, an inhibitor of tryptophan hydroxylase) or p-chloroamphetamine (PCA, a drug-inducing lesion of serotonergic nerve terminals). It is concluded that 8-OH-DPAT-induced increase in serum corticosterone concentration results from its action at a site different than the adrenal cortex and is mediated by postsynaptic 5-HT_{1A} receptors, whereas other subtypes (5-HT_{1B}, 5-HT_2, 5-HT_3) of 5-HT receptors do not participate in this response. Among other receptors only benzodiazepine ones are involved in the hormonal response to 8-OH-DPAT, whereas dopaminergic, α_2- and β-adrenergic as well as histamine and opiate receptors are not.

8-OH-DPAT 5-HT_{1A} receptor subtype 5-HT receptor antagonists Serum corticosterone

SEVERAL data suggest that serotonergic mechanisms are involved in regulation of corticosterone secretion. In fact, administration of 5-hydroxytryptamine (5-HT) precursors, releasers, reuptake inhibitors, or receptor agonists increases serum corticosterone levels (8, 9, 37). Furthermore, recent studies in this field demonstrated that two subtypes of 5-HT receptors, i.e., 5-HT_{1A} and 5-HT_2, mediate this hormonal response (20,24).

As to the involvement of 5-HT_{1A} receptors, the main evidence shows that 8-OH-DPAT, a selective agonist of these receptors (26), increases serum corticosterone and adrenocorticotropin hormone (ACTH) concentrations (10, 20, 24), and that these responses to 8-OH-DPAT are blocked by the drugs known to have 5-HT_{1A} antagonist properties (10,20).

However, as far as the role of 5-HT_{1A} receptors and the effect of 8-OH-DPAT are concerned, some other issues seem to deserve further examination. Thus, the evidence has been provided that 5-HT_{1A} receptors are located both pre- and postsynaptically (29,38), and that they mediate different responses to 8-OH-DPAT, e.g., hypothermia or hyperphagia and the stereotypical components of the serotonin syndrome, respectively (12, 13, 18, 36). Therefore, it seemed of interest to determine which 5-HT_{1A} receptors, i.e., pre- or postsynaptic ones, are involved in the corticosterone response to 8-OH-DPAT. Moreover, it has been recently reported that, besides 5-HT_{1A} receptors, α_2-adrenoceptors are also involved in some effects of 8-OH-DPAT, such as a decrease in 5-HT metabolism (25) and hyperglycemia and hypoinsulinemia (3) in rats. Since stimulation of α_2-adrenoceptors also increases ACTH and corticosterone secretion (32,33), examination of the role of these receptors in the 8-OH-DPAT-induced increase in the serum corticosterone level was another purpose of the present study. Finally, since in our experiments the response to 8-OH-DPAT was not fully blocked by 5-HT_{1A} receptor antagonists—even when administered in high doses—we also examined the influence of antagonists of other 5-HT receptor subtypes and other neurotransmitter receptors on that response.

METHOD

Male Wistar rats weighing 220–250 g were obtained from

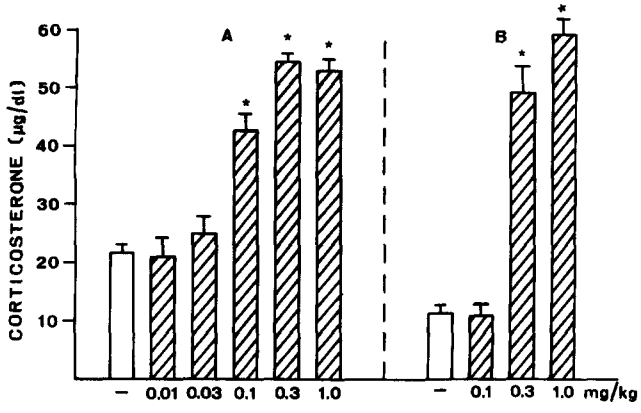

FIG. 1. Dose-response effect of 8-OH-DPAT on the serum corticosterone concentration in rats. 8-OH-DPAT was administered SC 30 min (A) or 60 min (B) before decapitation. Each bar is the mean ± S.E.M. of 10–12 animals. *$p<0.001$ vs. corresponding control group.

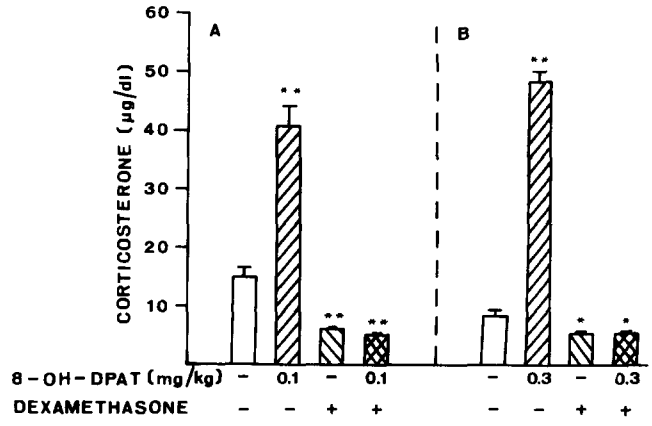

FIG. 3. The effect of 8-OH-DPAT on the serum corticosterone concentration in rats pretreated with dexamethasone. 8-OH-DPAT was administered SC 30 min (A) or 60 min (B) before decapitation. Dexamethasone (3 × 1 mg/kg) was injected IP 72, 48 and 24 hr before 8-OH-DPAT. Each bar is the mean ± S.E.M. of 10–12 animals. *$p<0.02$, **$p<0.001$ vs. corresponding control group.

licensed dealers. They were housed 5 animals to a cage for at least a week until use in the experiments, in a temperature-controlled room (22 ± 1°C) with a 12:12 light-dark cycle (lights on at 7.00 a.m.). Food and water were always available ad lib. All experiments were carried out between 9.00 and 11.00 a.m. to avoid the influence of the circadian rhythm on the serum corticosterone level.

In the first experiment, dose-effect and time-effect relationships were determined for 8-OH-DPAT-induced corticosterone secretion. 8-OH-DPAT (Research Biochemical Inc.) was dissolved in a solution of 0.1% sodium metabisulfite and administered subcutaneously (SC).

In subsequent experiments the effects of various drugs on 8-OH-DPAT-induced corticosterone secretion were determined. The following drugs were used: atenolol (ICI Pharma), DL-p-chloroamphetamine hydrochloride (PCA) (Regis Chemical Co.), DL-p-chlorophenylalanine methyl ester hydrochloride (PCPA) (Sigma Chemical Co.), chloropyramine (Polfa), dexamethasone (Sigma Chemical Co.), flumazenil (Ro 15-1788) (Hoffmann-La Roche), ICS 205-930 (Sandoz), idazoxan hydrochloride (Reckitt and Colman), ketanserin (Janssen Pharmaceutica), naloxone (Endo Laboratories), pimozide (Gedeon-Richter), (±)- and (−)-pindolol (Sandoz), pirenperone hydrochloride (Janssen Pharmaceutica), (±)-propranolol (ICI Pharma), 21009 (Sandoz), spiperone (Janssen Pharmaceutica), yohimbine hydrochloride (Sigma Chemical Co.). In another experiment the effect of the latter drug on the secretion of corticosterone induced by clonidine (hydrochloride, Boehringer Ingelheim) was also examined.

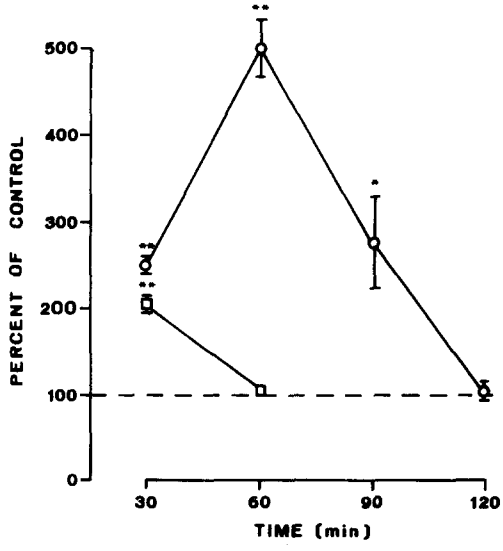

FIG. 2. Time-response effect of 8-OH-DPAT on the serum corticosterone concentration in rats. 8-OH-DPAT was administered SC in doses of 0.1 mg/kg (□) or 0.3 mg/kg (○). Values are the mean ± S.E.M. of 9–10 animals. Mean corticosterone concentrations in control rats were 24.4 ± 1.1 μg/dl (30 min) or 9.9 ± 1.1 μg/dl (60–120 min). *$p<0.01$, **$p<0.001$ vs. corresponding control group.

Ketanserin was dissolved in warm water. (±)- and (−)-pindolol were dissolved in a minimal quantity of 0.1 N hydrochloride acid and brought to the final volume with saline. Spiperone was dissolved in 0.1 M tartaric acid and titrated to pH 6 with 0.1 N sodium hydroxide. Yohimbine was dissolved in distilled water and diluted with saline. Dexamethasone, flumazenil, pimozide and 21009 were suspended in a 1% aqueous solution of Tween 80. The remaining drugs were dissolved in 0.9% saline. The drugs were administered SC or IP in a volume of 2 ml/kg at doses (expressed in terms of the respective salts) and times indicated in the Results section. Control rats received an equal volume of saline or appropriate solvent vehicle.

Rats were decapitated at different times after 8-OH-DPAT administration (30–120 min in time-effect relationship experiment and 30 and/or 60 min in all other experiments) and 30 min after clonidine injection. Trunk blood was collected and allowed to clot. After centrifugation, serum was withdrawn and stored at −20°C prior to analysis. Serum corticosterone level was measured spectrofluorometrically by the method of Glick *et al.* (11).

The statistical significance between groups was analysed by Student's *t*-test.

RESULTS

8-OH-DPAT administered 30 or 60 min before decapitation increased dose-dependently serum corticosterone concentration

TABLE 1

THE EFFECT OF 5-HT_{1A} RECEPTOR ANTAGONISTS ON SERUM CORTICOSTERONE CONCENTRATION INCREASED BY 8-OH-DPAT

Treatment	N	I μg/dl	%	N	II μg/dl	%
Vehicle + vehicle	8	15.2 ± 0.9	100	8	11.3 ± 2.0	100
Vehicle + 8-OH-DPAT	8	35.8 ± 2.6‡	236	9	53.1 ± 2.0‡	470
Spiperone (0.3)* + 8-OH-DPAT	8	32.7 ± 4.5†	215	9	43.5 ± 5.6‡	385
Spiperone (1.0) + 8-OH-DPAT	9	33.6 ± 3.5‡	221	9	45.2 ± 3.3‡	400
Spiperone (3.0) + 8-OH-DPAT	9	19.6 ± 4.0§	129	9	27.2 ± 4.0†¶	241
Vehicle + vehicle	9	15.2 ± 1.2	100	10	7.6 ± 0.9	100
Vehicle + 8-OH-DPAT	12	45.3 ± 2.6‡	298	9	42.9 ± 2.1‡	564
(±)-Pindolol (2.0) + 8-OH-DPAT	8	34.8 ± 1.8‡§	229	10	19.7 ± 2.3‡¶	259
(±)-Pindolol (4.0) + 8-OH-DPAT	11	30.4 ± 2.2‡¶	200	10	16.3 ± 2.6†¶	214
(±)-Pindolol (8.0) + 8-OH-DPAT	10	31.0 ± 2.5‡¶	204	10	22.1 ± 2.8‡¶	291
(−)-Pindolol (0.1) + 8-OH-DPAT	9	42.0 ± 3.6‡	276			
(−)-Pindolol (0.3) + 8-OH-DPAT	11	41.3 ± 3.4‡	272			
(−)-Pindolol (1.0) + 8-OH-DPAT	11	28.6 ± 2.9‡¶	188			
Vehicle + vehicle	8	19.1 ± 2.7	100			
Vehicle + 8-OH-DPAT	10	51.0 ± 3.5‡	267			
(±)-Propranolol (4.0) + 8-OH-DPAT	12	39.0 ± 3.1‡§	204			
(±)-Propranolol (16.0) + 8-OH-DPAT	9	35.7 ± 4.3‡§	187			

8-OH-DPAT was administered SC in a dose of 0.1 mg/kg 30 min before decapitation (I) or in a dose of 0.3 mg/kg 60 min before decapitation (II). Spiperone was administered IP 60 min before 8-OH-DPAT; (±)- and (−)-pindolol and (±)-propranolol were injected SC 30 min before 8-OH-DPAT. Values represent the mean ± S.E.M.

*mg/kg; †$p<0.01$, ‡$p<0.001$ vs. corresponding vehicle + vehicle group; §$p<0.05$, ¶$p<0.001$ vs. corresponding vehicle + 8-OH-DPAT group.

(Fig. 1). In a time-related experiment the peak effect of the drug was also dose-dependent, but the maximum effect after the higher dose appeared later. Thus, after a dose of 0.1 mg/kg, the maximum increase in corticosterone level (by ca. 100%) appeared 30 min after 8-OH-DPAT administration, and after a dose of 0.3 mg/kg (maximum increase by ca. 400%), 60 min after its administration (Fig. 2). On the basis of these results in the majority of further drug interaction experiments, 8-OH-DPAT was administered in a dose of 0.1 mg/kg (30 min before decapitation) or 0.3 mg/kg (60 min before decapitation).

Dexamethasone treatment significantly decreased serum corticosterone concentration and completely prevented 8-OH-DPAT-induced elevation of corticosterone level (Fig. 3).

TABLE 2

THE EFFECT OF 5-HT_{1B}, 5-HT_2 AND 5-HT_3 RECEPTOR ANTAGONISTS ON SERUM CORTICOSTERONE CONCENTRATION INCREASED BY 8-OH-DPAT

Treatment	N	I μg/dl	%	N	II μg/dl	%
Vehicle + vehicle	11	18.8 ± 2.2	100	11	10.6 ± 1.5	100
Vehicle + 8-OH-DPAT	9	41.7 ± 2.0†	222	12	46.4 ± 3.9†	438
21009 (2.0)* + 8-OH-DPAT				12	39.5 ± 4.2†	373
21009 (8.0) + 8-OH-DPAT	10	44.9 ± 1.9†	238	11	37.2 ± 3.0†	351
Vehicle + vehicle	9	16.6 ± 2.1	100	14	11.5 ± 1.2	100
Vehicle + 8-OH-DPAT	10	43.5 ± 2.8†	261	12	47.1 ± 4.2†	409
Ketanserin (2.0) + 8-OH-DPAT	11	50.7 ± 1.8†‡	305	10	43.0 ± 2.6†	374
Vehicle + vehicle	7	18.8 ± 1.4	100	12	9.2 ± 1.4	100
Vehicle + 8-OH-DPAT	10	42.1 ± 2.3†	224	12	46.4 ± 3.7†	504
Pirenperone (0.1) + 8-OH-DPAT	10	42.8 ± 2.2†	228	12	38.6 ± 2.9†	420
Pirenperone (0.2) + 8-OH-DPAT	10	46.1 ± 3.1†	245	11	43.2 ± 2.3†	470
Vehicle + vehicle	7	15.5 ± 1.2	100			
Vehicle + 8-OH-DPAT	11	49.0 ± 3.2†	316			
ICS 205-930 (3.0) + 8-OH-DPAT	12	58.2 ± 1.6†§	375			

8-OH-DPAT was administered SC in a dose of 0.1 mg/kg 30 min before decapitation (I) or in a dose of 0.3 mg/kg 60 min before decapitation (II). 21009 and ICS 205-930 were administered IP 30 or 60 min before 8-OH-DPAT, respectively. Ketanserin and pirenperone were injected SC 30 min before 8-OH-DPAT. Values represent the mean ± S.E.M.

*mg/kg; †$p<0.001$ vs. corresponding vehicle + vehicle group; ‡$p<0.05$, §0.02 vs. corresponding vehicle + 8-OH-DPAT group.

Spiperone (3 mg/kg), (±)-pindolol (2–8 mg/kg), (−)-pindolol (1 mg/kg) and (±)-propranolol (4 and 16 mg/kg) significantly attenuated the effect of 8-OH-DPAT on corticosterone secretion, though complete blockade of the effect was observed after spiperone versus the lower dose of 8-OH-DPAT only (Table 1). Lower doses of spiperone (0.3 and 1 mg/kg) and (−)-pindolol (0.1 and 0.3 mg/kg) were ineffective (Table 1).

Pretreatment of rats with 21009 (2 and 8 mg/kg), ketanserin (2 mg/kg), pirenperone (0.1 and 0.2 mg/kg) or ICS 205-930 (3 mg/kg) failed to attenuate the corticosterone response to 8-OH-DPAT (Table 2). On the contrary, a small but significant enhancement of the effect induced by a lower dose of 8-OH-DPAT was observed after ketanserin and ICS 205-930 (Table 2).

Yohimbine (0.125 and 0.5 mg/kg) and idazoxan (1 mg/kg) did not affect the hormone response to 8-OH-DPAT, though the former drug reduced dose-dependently clonidine-induced increase in serum corticosterone (Table 3).

As shown in Tables 4 and 5, 8-OH-DPAT-induced corticosterone secretion was not modified by pimozide (3 mg/kg), atenolol (10 mg/kg), chloropyramine (10 mg/kg), naloxone (10 mg/kg), PCA (2 × 10 mg/kg) or PCPA (3 × 150 mg/kg), but that effect was significantly, though only partially, reduced by flumazenil (15 mg/kg).

TABLE 3

THE EFFECT OF α_2-ADRENOCEPTOR ANTAGONIST ON SERUM CORTICOSTERONE CONCENTRATION INCREASED BY 8-OH-DPAT OR CLONIDINE

Treatment	N	I μg/dl	%	N	II μg/dl	%
Vehicle + vehicle	9	15.0 ± 1.5	100	8	6.0 ± 0.6	100
Vehicle + 8-OH-DPAT	8	40.8 ± 2.1†	272	8	52.4 ± 3.0†	866
Yohimbine (0.125)* + 8-OH-DPAT	10	38.6 ± 1.8†	257	8	49.1 ± 1.8†	811
Yohimbine (0.5) + 8-OH-DPAT	12	38.1 ± 1.8†	254	8	55.7 ± 3.8†	920
Vehicle + vehicle	10	24.1 ± 2.1	100	10	9.7 ± 0.6	100
Vehicle + 8-OH-DPAT	10	43.5 ± 4.2†	180	10	59.7 ± 3.4†	616
Idazoxan (1.0) + 8-OH-DPAT	11	55.0 ± 3.4†	228	10	58.8 ± 3.2†	606
Vehicle + vehicle	10	15.0 ± 1.5	100			
Vehicle + clonidine	11	41.6 ± 1.1†	277			
Yohimbine (0.125) + clonidine	12	30.1 ± 3.2†‡	201			
Yohimbine (0.5) + clonidine	11	15.1 ± 2.1§	101			

8-OH-DPAT was administered SC in a dose of 0.1 mg/kg 30 min before decapitation (I) or in a dose of 0.3 mg/kg 60 min before decapitation (II). Clonidine was administered SC in a dose of 0.1 mg/kg 30 min before decapitation. Yohimbine and idazoxan were injected SC or IP, respectively, 30 min before 8-OH-DPAT or clonidine. Values represent the mean ± S.E.M.

*mg/kg; †$p<0.001$ vs. corresponding vehicle + vehicle group; ‡$p<0.01$, §0.001 vs. vehicle + clonidine group.

TABLE 4

THE EFFECT OF VARIOUS RECEPTOR ANTAGONISTS ON SERUM CORTICOSTERONE CONCENTRATION INCREASED BY 8-OH-DPAT

Treatment	N	I μg/dl	%	N	II μg/dl	%
Vehicle + vehicle	9	14.4 ± 1.3	100	8	7.0 ± 1.1	100
Vehicle + 8-OH-DPAT	7	36.0 ± 3.4†	249	7	40.4 ± 4.6†	578
Pimozide (3.0) + 8-OH-DPAT	7	39.6 ± 3.0†	275	7	43.9 ± 2.6†	627
Vehicle + vehicle	10	24.4 ± 1.1	100	10	7.7 ± 1.0	100
Vehicle + 8-OH-DPAT	10	50.1 ± 2.5†	206	8	45.2 ± 2.1†	587
Atenolol (10.0) + 8-OH-DPAT	14	51.5 ± 1.6†	211	9	42.2 ± 2.9†	548
Vehicle + vehicle	7	15.5 ± 1.2	100			
Vehicle + 8-OH-DPAT	11	49.0 ± 3.2†	316			
Chloropyramine (10.0) + + 8-OH-DPAT	13	46.6 ± 2.9†	301			
Vehicle + vehicle	10	23.2 ± 2.6	100	10	11.8 ± 1.5	100
Vehicle + 8-OH-DPAT	11	41.7 ± 1.6†	180	9	47.1 ± 2.2†	399
Naloxone (10.0) + 8-OH-DPAT	12	40.7 ± 2.3†	175	9	38.2 ± 3.8†	324
Vehicle + vehicle	8	11.9 ± 1.7	100	8	7.9 ± 0.8	100
Vehicle + 8-OH-DPAT	8	48.3 ± 4.0†	405	7	49.3 ± 2.8†	621
Flumazenil (15.0) + 8-OH-DPAT	9	30.8 ± 4.7†	259	8	32.3 ± 5.2†‡	407

8-OH-DPAT was administered SC in a dose of 0.1 mg/kg 30 min before decapitation (I) or in a dose of 0.3 mg/kg 60 min before decapitation (II). The antagonists were injected IP in following times before 8-OH-DPAT: naloxone—5 min, atenolol and flumazenil—30 min, pimozide and chloropyramine—60 min. Values represent the mean ± S.E.M.

*mg/kg; †$p<0.001$ vs. corresponding vehicle + vehicle group; ‡$p<0.02$ vs. corresponding vehicle + 8-OH-DPAT group.

The examined drugs (except dexamethasone) whose interaction with 8-OH-DPAT was studied, in the doses used, did not affect by themselves the serum corticosterone level (results not shown).

DISCUSSION

In accordance with the recent findings of Koenig *et al.* (20) and Lorens and Van de Kar (24), the results of the present study demonstrate that 8-OH-DPAT, a selective 5-HT_{1A} receptor agonist (26), increases the serum corticosterone concentration. Since the formation and secretion of corticosterone from the adrenal glands is stimulated by the anterior pituitary peptide ACTH, whose release is controlled by the hypothalamic corticotropin-releasing hormone, the above effect of 8-OH-DPAT indicates activation of the hypothalamic-pituitary-adrenocortical axis.

To exclude the possibility of a direct effect of 8-OH-DPAT on the adrenal glands, we studied its influence on corticosterone secretion in rats pretreated with dexamethasone. Since the synthetic corticoid completely blocked the 8-OH-DPAT-induced effect, it might be concluded that the corticosterone response to 8-OH-DPAT results from its action at the level superior to the adrenal glands, i.e., at the hypothalamo-pituitary level. This conclusion is in line with the results recently reported by Gilbert *et al.* (10), who found that 8-OH-DPAT increased the rat plasma ACTH concentration. However, since the effect of the drug on the release of the hypothalamic corticotropin-releasing hormone is unknown, and since 5-HT and its agonists can directly stimulate the ACTH release from the anterior pituitary (34), the exact site of action of 8-OH-DPAT at the hypothalamo-pituitary level remains unclear. Moreover, the results of studies into distribution of 5-HT receptor subtypes, which might be expected to throw some light on this problem—on the assumption that the hormonal response to 8-OH-DPAT is mediated via 5-HT_{1A} receptors (see below)—do not contribute to solving it. Actually, Pazos and Palacios (29) reported that in different nuclei of the hypothalamus, the 5-HT_{1A} subtype is only a small part of the whole population of 5-HT_1 receptors (most of which exhibit characteristics of the 5-HT_{1B} subtype). Furthermore, in the pituitary (including the anterior lobe), 5-HT_2 receptors were identified, 5-HT_1 ones not having been examined though (4).

The evidence that 8-OH-DPAT-induced elevation in serum corticosterone level results from stimulation of 5-HT_{1A} receptors was presented by Koenig *et al.* (20), who found that this response was blocked by spiperone and pindolol which bind with a relatively high affinity to 5-HT_{1A} sites (5,17). Accordingly, we found that the response to 8-OH-DPAT was antagonized by the two drugs mentioned above as well as by propranolol which also binds to 5-HT_{1A} receptors (5,17). Although spiperone is also an antagonist of dopaminergic and 5-HT_2 receptors (22), whereas pindolol and propranolol are β-blockers with a high affinity to the 5-HT_{1B} sites (5,17), involvement of these receptors in the response to 8-OH-DPAT seems unlikely. In fact, we demonstrated

TABLE 5

THE EFFECT OF p-CHLOROAMPHETAMINE (PCA) AND p-CHLOROPHENYLALANINE (PCPA) ON SERUM CORTICOSTERONE CONCENTRATION INCREASED BY 8-OH-DPAT

Treatment	I N	I μg/dl	I %	II N	II μg/dl	II %
Vehicle + vehicle	6	22.2 ± 4.1	100	6	12.5 ± 1.0	100
Vehicle + 8-OH-DPAT	11	50.5 ± 3.5*	228	11	51.9 ± 4.7*	415
PCA + 8-OH-DPAT	14	45.0 ± 1.5*	203	15	48.2 ± 1.7*	386
Vehicle + vehicle	7	22.2 ± 4.1	100	6	14.6 ± 2.4	100
Vehicle + 8-OH-DPAT	12	46.1 ± 2.9*	208	11	50.7 ± 2.7*	347
PCPA + 8-OH-DPAT	13	46.2 ± 2.8*	208	11	45.4 ± 3.5*	311

8-OH-DPAT was administered SC in a dose of 0.1 mg/kg 30 min before decapitation (I) or in a dose of 0.3 mg/kg 60 min before decapitation (II). PCA (2×10 mg/kg, 192 and 168 hr before 8-OH-DPAT) and PCPA (3×150 mg/kg, 72, 48 and 24 hr before 8-OH-DPAT) were injected IP. Values represent the mean ± S.E.M.

*$p<0.001$ vs.corresponding vehicle + vehicle group.

that the response was not attenuated by pimozide, a dopaminergic receptor antagonist with a low affinity to 5-HT receptors (22), and the selective 5-HT_2 receptor antagonists ketanserin and pirenperone (14,23); the latter results are in accordance with those of Koenig *et al.* (20), who found no effect of ketanserin and the other two 5-HT_2 receptor antagonists altanserin and ritanserin. Furthermore, we also observed the lack of antagonistic effects of atenolol, a β-adrenoceptor blocker with no affinity to 5-HT_{1A} and 5-HT_{1B} receptors (16), and 21009, a selective 5-HT_{1B} antagonist (7), having excluded participation of β-adrenoceptors and 5-HT_{1B} receptors. All these results, together with the lack of antagonistic effect of ICS 205-930, a selective antagonist of 5-HT_3 receptors (7), supply evidence indicating involvement of 5-HT_{1A} receptors in the hormonal response induced by 8-OH-DPAT.

However, if our results on 5-HT_{1A} receptor antagonists are compared with those of Koenig *et al.* (20), there is a noticeable difference. The latter authors demonstrated that corticosterone secretion, induced by 0.1 mg/kg of 8-OH-DPAT, was completely blocked by spiperone and (−)-pindolol administered in doses of 3 and 0.3 mg/kg, respectively. In our experiment those antagonists were not so effective. Having induced the hormonal response using 0.1 or 0.3 mg/kg (submaximal dose) of 8-OH-DPAT, we found that 3 mg/kg of spiperone fully antagonized the effect evoked by the lower dose of the agonist only, while (−)-pindolol, even in a dose of 1 mg/kg, was only partly effective towards the lower dose of 8-OH-DPAT. Furthermore, only partial antagonism was observed after (±)-pindolol (against both doses of 8-OH-DPAT) and (±)-propranolol (against the lower dose of the agonist), given in doses as high as 8 and 16 mg/kg, respectively. The results obtained with (±)-pindolol differ from those of Gilbert *et al.* (10), who reported that a dose of 4 mg/kg of the drug was sufficient to block completely another hormonal response (ACTH secretion) induced by 0.3 mg/kg of 8-OH-DPAT. The reason for these discrepancies is unclear the more so as our experimental conditions were comparable with those of Koenig *et al.* (20) and Gilbert *et al.* (10). However, one essential difference should be pointed out as they performed their experiments on Sprague-Dawley rats, while we used Wistar ones. Therefore, if the strain difference is responsible for the above discrepancies, it may also be postulated that in Wistar rats the corticosterone response to 8-OH-DPAT (especially to higher doses of the drug) is only partly mediated by 5-HT_{1A} receptors, and in part by other neurotransmitter receptor(s).

To prove this working hypothesis, we studied the influence of various receptor antagonists on the corticosterone response to 8-OH-DPAT. First of all, we examined the influence of α_2-adrenoceptor antagonists, since it was reported that these receptors mediate some other responses to 8-OH-DPAT, and since stimulation of α_2-adrenoceptors leads to corticosterone secretion (see Introduction). However, neither yohimbine nor idazoxan modified the effect of 8-OH-DPAT. Though yohimbine was used in relatively low doses—as those higher than 0.5 mg/kg induce by themselves increase in serum corticosterone (our unpublished results) or ACTH (32) concentrations—they were high enough to block efficiently the elevation of corticosterone level induced by the α_2-adrenoceptor agonist clonidine. Thus, involvement of these receptors may be excluded.

Furthermore, though central histamine and opiate receptors are also thought to play some role in regulation of corticosterone secretion (37), it is unlikely that 8-OH-DPAT induces increases in the hormone concentration via an interaction with any of these receptors, since neither the histamine (H_1) receptor antagonist chloropyramine nor the opiate receptor blocker naloxone altered the response to 8-OH-DPAT. It is noteworthy that naloxone was administered in the dose that was shown to block morphine-induced corticosterone secretion (35), and the used dose of chloropyramine was equivalent to doses of other histamine (H_1) receptor antagonists, which prevented ACTH response to histamine (31).

On the other hand, a partial but significant reduction of the 8-OH-DPAT-induced effect observed in animals pretreated with flumazenil, an antagonist of benzodiazepine receptors (1), indicates that a direct or indirect interaction between 8-OH-DPAT and these receptors may be involved. In this context it is noteworthy that 8-OH-DPAT induces antianxiety effects in experimental models (2,6), and that the classical anxiolytics benzodiazepines elevate the serum corticosterone level (27,30), this effect being sensitive to flumazenil (27).

Our other results indicate that 5-HT_{1A} receptors, which mediate the hormonal response to 8-OH-DPAT, are located postsynaptically. In fact, in rats in which 5-HT synthesis was inhibited by means of PCPA (19), as well as in animals whose 5-HT nerve terminals were lesioned by PCA (21,28)— both drugs being given in doses which reduce the whole brain 5-HT concentration by about 85–90% (results not shown)—the corticosterone response to 8-OH-DPAT was not affected. These results exclude involvement of presynaptic 5-HT_{1A} receptors located on 5-HT cell bodies or dendrites (38). Such a conclusion is also in line with the general concept that corticosterone secretion is caused by activation of 5-HT system (see Introduction), since stimulation of the presynaptic 5-HT_{1A} receptors by 8-OH-DPAT results in inhibition of the 5-HT synthesis and release (15,18).

In conclusion, the results of this study indicate that, in the rat, 8-OH-DPAT-induced increase in serum corticosterone concentration results from its action at the hypothalamo-pituitary level and is partly mediated by postsynaptic 5-HT_{1A} receptors, whereas other subtypes (5-HT_{1B}, 5-HT_2, 5-HT_3) of 5-HT receptors do not participate in this response. Among other receptors only benzodiazepine ones are involved in the hormonal response to 8-OH-DPAT, whereas dopaminergic, α_2- and β-adrenergic, as well as histamine (H_1) and opiate receptors, are not.

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