NOTES

Functional activity in the lateral habenular and dorsal raphe nuclei following administration of several dopamine receptor antagonists[1]

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2-[^{14}C]deoxyglucose autoradiography was used to show regional functional activity in the rat brain following administration of the dopamine antagonists, *cis*-flupenthixol, metoclopramide, and pimozide. Elevation of functional activity was observed in the lateral habenula (LHb) following administration of all antagonists. The dorsal raphe nucleus (DR) exhibited decreased functional activity following *cis*-flupenthixol, which exerts effects at both the D_1 and D_2 receptors, but not following pimozide or metoclopramide, which primarily act at the D_2 receptor. These results converge with previous reports suggesting that the functional status of the LHb is regulated by dopaminergic systems, and that LHb efferents may exert a major influence upon DR neurons. These data further suggest that an influence of the LHb upon DR neurons may be affected via the D_1 receptor.

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On a utilisé une autoradiographie au 2-[^{14}C]désoxyglucose pour montrer l'activité fonctionnelle régionale dans le cerveau de rat après l'administration des antagonistes dopaminergiques, *cis*-flupenthixol, métoclopramide et pimozide. On a observé une élévation de l'activité régionale dans l'habenula latérale (LHb) après l'administration de tous les antagonistes. Le noyau du raphé dorsal (DR) présenta une diminution d'activité fonctionnelle après l'administration de *cis*-flupenthixol; ce dernier exerçe ses effets tant au récepteur D_1 que D_2. On n'observa pas de diminution d'activité fonctionnelle après l'administration de pimozide ou de métoclopramide; ces derniers agissent principalement au récepteur D_2. Ces résultats rejoignent ceux de comptes rendus antérieurs suggérant d'une part, que le statut fonctionnel de la LHb est régi par des systèmes dopaminergiques et d'autre part, que les ganglions efférents de la LHb peuvent exercer une influence majeure sur les neurones du DR. Ces résultats suggèrent en outre que la LHb peut exercer une influence sur les neurones du DR via le récepteur D_1.

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The 2-[^{14}C]deoxyglucose technique (Sokoloff et al. 1977) has often been used to map levels of regional functional activity following manipulation of dopamine (DA) systems. A consistent finding is that the lateral habenular nucleus (LHb) exhibits decreased functional activity following administration of the DA agonist amphetamine or dopamine itself (Brown and Wolfson 1983), and increased functional activity following administration of DA antagonists (Gomita and Gallistel 1982; McCulloch et al. 1980) or DA denervation with 6-hydroxydopamine (Kozlowski and Marshall 1980; Wooten and Collins 1981). These findings suggest that the functional status of the LHb may be influenced by dopaminergic systems.

We have examined the effects of three DA antagonists on relative functional activity (RFA) in the LHb and on RFA in regions of the brain receiving major DA efferents. The antagonists were selected on the basis of their ability to differentially block subclasses of DA receptors (Kebabian and Calne 1979). Thus, *cis*-flupenthixol is a potent antagonist of both D_1 receptors (those linked to adenylate cyclase) and D_2 receptors (Hyttel 1982). Pimozide is a potent D_2 antagonist with weak effects at the D_1 receptor and metoclopramide is a D_2 antagonist with minimal effects on the D_1 receptor (Peringer et al. 1976; Seeman 1981).

Methods

Male Wistar rats (250–300 g, n = 3/group) received an ip injection of *cis*-flupenthixol (1.5 mg/kg), metoclopramide (10 mg/kg), pimozide (1.5 mg/kg), or saline. Under halothane anesthesia, the tail vein was cannulated. The region around the cannula was treated with a local anesthetic (Xylocaine). Halothane was discontinued, and the animals were wrapped with a towel for warmth. They were then placed in a Plexiglas rat restrainer. Three hours later, the alert animals were injected with 50 μCi/kg (1 Ci = 376 GBq) of 2-[^{14}C]deoxyglucose (59 μCi/mmol, Pathfinder) via the venous cannula. Following a 45-min isotope incubation period, the animals were sacrificed with a lethal dose of sodium pentobarbitol, the brains were extracted and cut at 20 μm, and autoradiographs were prepared on Kodak X-TL film. Autoradiographic optical densities in 50 brain regions including the LHb and 14 other regions with heavy DA innervation were quantified by computerized densitometry (Ramm and Kulick 1984).

Optical density values read from the autoradiographs were converted to tissue equivalent isotope concentration in underlying tissue (reflecting regional functional activity), using a calibrated (microcuries per gram) set of methacrylate ^{14}C standards (Amersham) exposed with each film. This procedure corrects for the nonlinear response of film to underlying radioactivity. Data were then normalized to a within-animal reference value to derive a measure of relative functional activity. The reference value used was mean isotope concentration in the 50 regions sampled. As mean isotope concentration was unaffected by any drug treatment ($p > 0.05$), there was no interaction between treatment group and mean isotope concentration to bias the normalization procedure. Further, the ratio of regional

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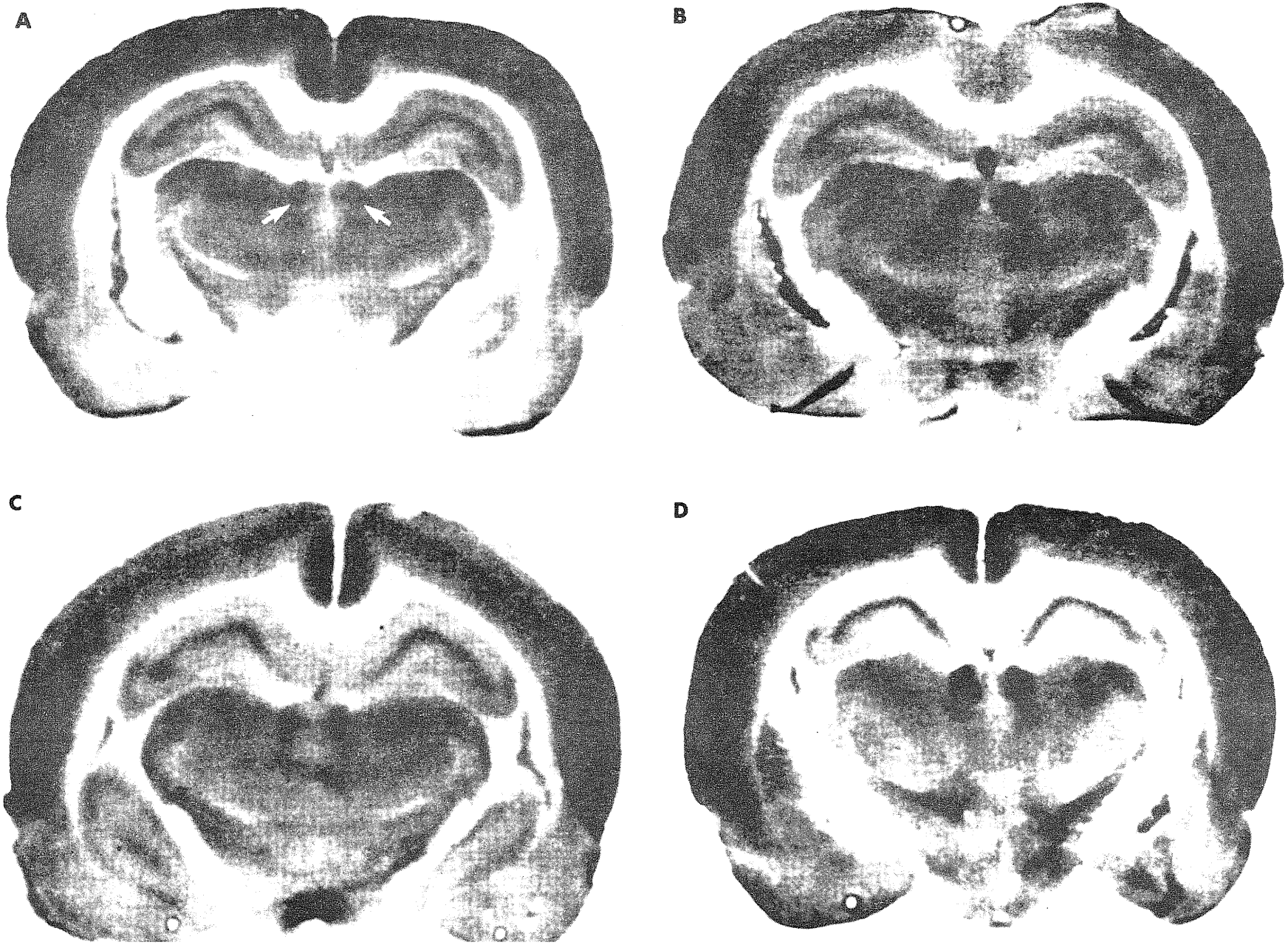

FIG. 1. Digitized autoradiographs of rat brain following administration of (A) saline, (B) pimozide, (C) metoclopramide, and (D) *cis*-flupenthixol. Arrows point to lateral habenula.

TABLE 1. Effects of pimozide (PIM), *cis*-flupenthixol (FLU), metoclopramide (MET), mean of all drugs (drug mean), and saline (SAL) upon relative functional activity (local functional activity/mean brain activity) in the rat brain

Brain region	PIM	FLU	MET	Drug mean	SAL
Anterior cortex	1.02(0.08)	1.08(0.09)	0.98(0.14)	1.03(0.10)	1.06(0.25)
N. accumbens	1.07(0.07)	0.99(0.15)	1.01(0.11)	1.02(0.22)	0.94(0.16)
Central amygdaloid n.	0.68(0.12)	0.78(0.21)	0.54(0.10)	0.67(0.05)	0.55(0.03)
Caudate putamen	1.16(0.06)	1.01(0.15)	1.12(0.20)	1.10(0.15)	0.95(0.24)
Dorsal raphe	1.05(0.05)	0.79(0.07)*	1.01(0.07)	0.95(0.13)	1.02(0.20)
Globus pallidus	0.54(0.05)	0.59(0.08)	0.52(0.07)	0.54(0.06)	0.47(0.05)
Lateral habenula	1.94(0.21)*	1.92(0.32)*	2.34(0.31)*	2.07(0.32)*	1.35(0.13)
Medial habenula	0.87(0.08)	0.85(0.07)	0.97(0.12)	0.89(0.10)	0.75(0.05)
Mesencephalic raphe n.	1.33(0.19)	1.15(0.17)	1.16(0.07)	1.21(0.16)	1.09(0.30)
Substantia nigra zone compacta	0.96(0.01)	0.87(0.12)	0.98(0.02)	0.94(0.08)	0.90(0.05)
Substantia nigra zone reticulata	0.63(0.13)	0.63(0.05)	0.67(0.03)	0.64(0.08)	0.60(0.11)
Ventral pallidum	0.64(0.04)	0.65(0.11)	0.57(0.06)	0.62(0.07)	0.56(0.01)
Ventral tegmental area	0.82(0.04)	0.83(0.14)	0.84(0.05)	0.83(0.08)	0.75(0.07)

NOTE: n, nucleus.
*$p < 0.05$ (Dunnett's test).

functional activity to mean brain functional activity has more intuitive meaning (see Ramm and Frost 1983) than does the more commonly used gray matter − white matter normalization procedure.

Results and discussion

The LHb exhibited elevated RFA ($p < 0.05$) under all three drug treatments (Table 1; Fig. 1). In addition, the *cis*-flupenthixol group showed a significant decrease of RFA in the dorsal raphe nucleus (DR) ($p < 0.05$). When all treated animals were combined for comparison with controls, the LHb again exhibited increased RFA ($p < 0.01$), but no other region exhibited a significant alteration.

The reciprocal pattern of RFA in the DR and LHb is in agreement with reports that habenular stimulation suppresses the firing of raphe units (Stern et al. 1979; Wang and Aghajanian 1977). The anatomical substrate of the metabolic and electrophysiological effects may lie in the habenulo-raphe projection, the major source of forebrain projections to the midbrain raphe (Aghajanian and Wang 1977; Akagi and Powell 1968; Herkenham and Nauta 1979; Pasquier et al. 1976). In sum, electrophysiological, anatomical, and now functional data suggest that LHb efferents may exert a major influence upon the activity of mesencephalic raphe neurons. The significant effect of *cis*-flupenthixol but not pimozide or metoclopramide upon DR RFA suggests that D_1 receptors may be importantly involved.

The LHb is a point of convergence for DA nigral efferents and for fibers connecting forebrain limbic regions via the dorsal diencephalic conduction system. In turn, LHb efferents terminate in numerous mesencephalic sites. These include the ventral tegmentum, substantia nigra, central gray, mesencephalic reticular formation, and mesencephalic raphe nuclei (Sutherland 1982). As the LHb is a probable site of interaction between ascending DA systems and the limbic forebrain and midbrain (Herkenham and Nauta 1977; McCulloch et al. 1980; Phillipson and Griffith 1980; Sutherland 1982; Wang and Aghajanian 1977), it is also possible that activity in this relay links the motor and emotional effects of manipulation of DA systems. Thus, the effects of DA antagonism upon metabolic activity in the LHb may reflect functional interaction between limbic and motor systems.

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