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Are dopamine receptors present on human lymphocytes?

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The characteristics of [^{3}H]spiperone binding to human lymphocytes have been examined. The haloperidol displaceable component of [^{3}H]spiperone binding to human lymphocytes was not saturable, and stereoselective displacement by the isomers of butaclamol was not observed. There was no correlation between the ability of known dopamine active drugs to cause displacement of the ligand and their rank order of potency. Chloroquine, a drug with no dopaminergic action, was the most potent displacing agent examined. Fragmentation of the cells caused a marked decrease in the haloperidol displaceable component of [^{3}H]spiperone binding, and saturable binding to the lymphocyte membrane fragments was not observed. Lysis of cells after equilibrium incubation with [^{3}H]spiperone caused a marked reduction in the haloperidol displaceable component of ligand binding. Association of [^{3}H]spiperone with lymphocytes was unaffected by the metabolic inhibitor iodoacetate, or by replacement of sodium ions by lithium ions in the incubation medium, suggesting that such association did not involve an active uptake process. We cannot confirm the existence of dopamine receptors on the surface of human lymphocytes. We suggest that the apparent association of [^{3}H]spiperone with lymphocytes is due to some passive uptake process causing accumulation of the ligand within the cells.

A peripheral model of cerebral dopamine receptor function in man would greatly aid pathological investigations of clinical neurological and psychiatric disorders. Recently, specific binding of the dopamine antagonist ligand [^{3}H]spiperone to rat lymphocytes was reported to exhibit characteristics identical to those of dopamine receptors found in rat striatal preparations (Le Fur et al 1980). Thus, the specific binding of [^{3}H]spiperone was saturable and was stereospecifically displaced by the isomers of butaclamol. The relative potencies of a range of dopamine active compounds in displacing [^{3}H]spiperone paralleled their activity in displacing this ligand from striatal preparations. Using the procedure described for rat lymphocytes, a saturable binding site on human lymphocytes was also described, although no detailed characterization was presented (Le Fur et al, 1980). Subsequently, these workers reported a decreased number of specific [^{3}H]spiperone binding sites on lymphocytes from untreated parkinsonian patients, and a restoration to control levels by levodopa therapy (Le Fur et al 1981 a,b). These results suggested that the binding of [^{3}H]spiperone to lymphocytes might provide a viable model of changes in cerebral dopamine receptor function. Accordingly, we have tried to establish this technique in our laboratories and have carried out a detailed examination of [^{3}H]spiperone binding to human lymphocyte preparations. We are unable to confirm, however, the existence of dopamine receptors on human lymphocytes.

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MATERIALS AND METHODS

Incubation of [^{3}H]spiperone with human lymphocytes

Lymphocytes were prepared from 60 ml samples of venous blood collected from healthy volunteers aged 20–40 years using a Ficoll-Pacque gradient and Hank's medium (Wellcome) as previously described (Boyum 1967; Fotino et al 1971). The lymphocyte preparation was diluted with Hank's medium such that 1 ml contained 10^7 cells. Viability was assessed by exclusion of trypan blue dye.

Specific binding of [^{3}H]spiperone (0.2–20 nM; 25 Ci mmol; Amersham International), was determined as follows: aliquots of the lymphocyte suspension (0·1 ml containing 10^6 cells) were pre-incubated for 10 min at room temperature following the addition of either a displacing drug (0·05 ml) dissolved in 0·1% ascorbic acid solution or 0·1% ascorbic acid (0·05 ml) alone. Following the addition of [^{3}H]spiperone (0·2–20 nM in 0·05 ml Hank's medium) samples were incubated in a shaking water bath for 1 h at 37 °C. At the end of this period ice-cold 50 mM Tris-HCl buffer, pH 7·5, containing 100 mM NaCl (3 ml) was added and samples immediately filtered through

GF/C Whatman filters using a Millipore vacuum filtration apparatus. The filters were washed 3 times with 50 mM Tris-HCl buffer, pH 7·5, containing 100 mM sodium chloride (3 ml) and transferred to vials containing Instagel (Packard) liquid scintillation cocktail (10 ml). Vials were shaken vigorously to disrupt the filters and subjected to liquid scintillation counting at an efficiency of between 46–50%. At each ligand concentration or at each concentration of displacing agent, analysis was always carried out in triplicate. Displaceable binding was defined as that occurring in the presence and absence of either 10^{-5} M haloperidol (Janssen Pharmaceutica) or 10^{-5} M (+)-butaclamol (Ayerst Laboratories).

In a typical experiment using 4 nm [^{3}H]spiperone, 16 000 counts min^{-1} of standard were added, total binding was 2400 counts min^{-1}, non-specific binding in the presence of 10^{-5} M haloperidol was 1050 counts min^{-1} and filter binding was 310 counts min^{-1}.

Drug displacement experiments

For the determination of the relative ability of drugs to displace [^{3}H]spiperone binding to lymphocyte preparations a single ligand concentration (4 nM) was employed. Drugs were incorporated into incubates in a range of concentrations between 10^{-10}–10^{-3} M. All drugs were dissolved in 0·1% ascorbic acid. The following compounds were employed: (+)- or (−)-butaclamol hydrochloride (Ayerst); haloperidol, spiperone, pimozide, domperidone, R 41468 (3-[2-[4-(fluorobenzoyl)-1-piperidinyl]ethyl]2,4-(1H,3H)- quinazolinedione) R 5260 8-(4-oxo-3,3-diphenylhexyl)-1-phenyl-1,3,8-triazospiro[4.5]decan-4-one, R 5573 8-(3,3-diphenylpropyl)-1-phenyl-1,3,8-triazospiro[4.5]decan-4-one (all Janssen); *cis*-flupenthixol (Lundbeck) phenoxybenzamine dihydrochloride (*SKF*), propranolol hydrochloride (ICI), clonidine hydrochloride (Boehringer), sulpiride (Delagrange) dihydroergocriptine methane sulphonate (Sandoz), and 5-hydroxytryptamine creatine sulphate and dopamine hydrochloride (Sigma) and chloroquine phosphate (Winthrop).

Incubation of [^{3}H]spiperone with lymphocyte membranes

In some experiments the binding of [^{3}H]spiperone to lymphocyte membrane fragments was examined. Two techniques were used for the preparation of membranes: (a) aliquots of the final lymphocyte preparation were frozen at −20 °C and then thawed, this procedure being repeated three times. Cells were then homogenized using a Polytron homogenizer (setting 5) for 10 s. (b) aliquots of the final lymphocyte preparation were suspended in hypotonic 5 mM Tris-HCl buffer, pH 7·4, for 5 min and then centrifuged at 4000*g* for 30 min. The resulting pellet was suspended in Hank's medium by homogenization (Polytron setting 5) for 10 s.

The effect of cell lysis on the haloperidol displaceable compound of [^{3}H]spiperone binding

In a further series of experiments, we investigated the effect of cell lysis on the binding of [^{3}H]spiperone to intact lymphocyte preparations. Cell preparation and incubation with [^{3}H]spiperone was as described in the general methodology above. After 60 min incubation at 37 °C we added to each sample either 3 ml of a hypotonic ice-cold solution of 5 mM Tris-HCl, pH 7·4, containing 5 mM sodium chloride or 3 ml isotonic ice-cold 140 mM sucrose, 5 mM Tris-HCl, buffer, pH 7·4, containing 5 mM sodium chloride. Samples were allowed to stand for 2 min after which they were filtered and washed as above, except that GF/B filters were employed to ensure trapping of membrane fragments.

Inhibition of active uptake processes

To evaluate the role of active uptake processes in the association of [^{3}H]spiperone with human lymphocyte preparations, samples were preincubated with iodoacetate (1 mM) for 30 min at 37 °C. Total and haloperidol (10^{-5} M) displaceable binding then was determined as described above. In addition other lymphocytes were suspended in buffer where sodium chloride was replaced by lithium chloride (120 mM). Total and haloperidol (10^{-5} M) displaceable binding again was determined.

RESULTS

Lymphocyte preparations

Viability of human lymphocyte preparations was always greater than 95%. Examination, using Leishmann staining, of the final cell suspension showed contamination with less than 5% polymorphonuclear cells and red blood cells. There was approximately one platelet to every two lymphocytes in final cell preparations. However, analysis of [^{3}H]spiperone binding to pure platelet preparations showed that this contamination would account for less than 5% of the [^{3}H]spiperone binding to lymphocyte suspensions (unpublished observations).

Incubation of lymphocytes with [^{3}H]spiperone and drug displacement studies

Total binding of [^{3}H]spiperone (0·2–20 nM) was

linear over the concentration range employed. The inclusion of a high concentration of haloperidol or (+)-butaclamol (10^{-5} M) caused a 60–70% displacement of total binding at all ligand concentrations. The displaceable component was proportional to the [^{3}H]spiperone concentration such that 15–25 fmol/10^6 cells/nM [^{3}H]spiperone were bound. There was no evidence of saturation of specific [^{3}H]spiperone binding (Fig. 1A).

The isomers of butaclamol (10^{-9}–10^{-4} M) were equipotent in their ability to displace [^{3}H]spiperone from its binding site on the lymphocyte preparation (Fig. 1B).

The ability of a range of neuroleptic drugs to displace the binding of [^{3}H]spiperone showed the IC50 values to be far in excess of the concentration required to displace [^{3}H]spiperone from specific binding sites on striatal preparations (Table 1). Also, the order of potency for such displacement of [^{3}H]spiperone from lymphocytes differed from that accepted for action at dopamine receptors, such as those on striatal preparations (Table 1). In addition, 5-HT, the specific 5-HT antagonist R 41468, phenoxybenzamine, propranolol, clonidine and dihydroergocriptine only weakly displaced [^{3}H]spiperone from lymphocytes. R 5537 and R 5260, which are spirodecanone compounds and structural analogues of spiperone and which might be expected to displace [^{3}H]spiperone from a saturable non-specific spirodecanone site (see Howlett et al 1979), were among the most potent displacing agents, but even these were only weakly effective. Chloroquine, a lysosomotropic agent with no dopaminergic actions, was the most potent of the displacing agents examined.

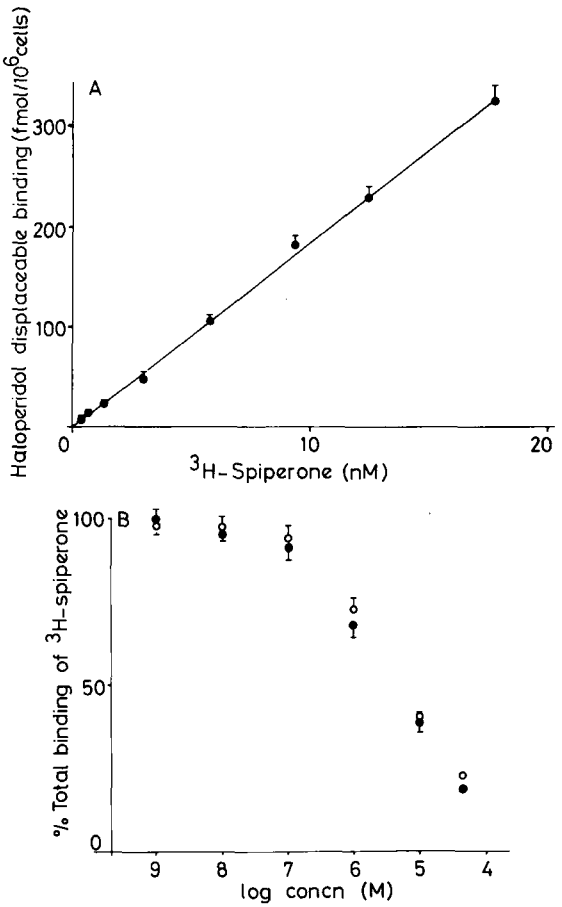

FIG. 1. The effect of (A) increasing [^{3}H]spiperone (0·2–20 nM) concentrations on the haloperidol (10^{-5} M) displaceable component of binding and (B) the incorporation of (+)- butaclamol (-●-) or (−)-butaclamol (-○-) (10^{-9}–10^{-4} M), on the total binding of [^{3}H]spiperone (4 nM) to lymphocyte preparations. Each point is the average of 2 (in A) and 4 (in B) separate experiments carried out in triplicate at each ligand concentration, or at each concentration of displacing agent, and using separate lymphocyte preparations.

Binding of [^{3}H]spiperone to lymphocyte membrane preparations

The total binding of [^{3}H]spiperone to lymphocyte membrane preparations prepared by either freezing

Table 1. Displacement of [^{3}H]spiperone (4nM) from human lymphocytes by drugs acting at dopamine, noradrenaline and 5-hydrotryptamine receptors, compared with the displacement of the same ligand from dopamine receptors in striatal preparations.

	IC50 (nM)	
Displacing agent	Lymphocytes	Striatal preparations
Chloroquine	220 (±120)	7600[e]
cis-Flupenthixol	300 (±110)	59[a]
R 5537	410 (± 80)	—
Haloperidol	600 (±220)	20[a]
R 5260	1100 (±300)	—
(+)-Butaclamol	1100 (±380)	20[a]
(−)-Butaclamol	1300 (±400)	10 000[a]
Spiperone	2500 (±500)	1·3[a]
R 41468	1100 (±260)	550[b]
Domperidone	10 000 (±2600)	4·5[c]
Dihydroergocriptine	14 000 (±3800)	68[c]
Clonidine	40 000	10 000[d]
Phenoxybenzamine	> 10 000	500[b]
Propranolol	> 10 000	10 000[d]
Sulpiride	>100 000	880[a]
5-HT	126 000 (± 40 000)	80 000[a]
Dopamine	530 000 (±120 000)	6400[a]

Each value is the mean of two determinations made on separate lymphocyte preparations. Drugs were included in concentrations between 10^{-10}–10^{-3} M and examined in triplicate at each concentration.

[a] Leysen et al (1978), [b] Leysen et al (1981), [c] Seeman (1980), [d] Andorn & Maguire (1980), [e] Maloteaux et al (1982).

or hypnotic shock was only 20% of that occurring to intact lymphocyte cells (Table 2). Virtually none of the [^{3}H]spiperone associated with membrane preparations was displaced by the incorporation of haloperidol (10^{-5} M).

Table 2. The total and haloperidol (10^{-5} M) displaceable components of [^{3}H]spiperone (4 nM) binding to human lymphocyte membranes prepared by either freezing and subsequent homogenization, or by hypotonic shock and subsequent homogenization (see methods) compared with binding to intact cells.

	[^{3}H]Spiperone bound (fmol/10^6 cells/nM)		
		Lymphocyte membranes	
	Intact cells	Freezing	Hypotonic shock
Total binding	28 ± 3	6·7 ± 0·8	5·7 ± 1
Haloperidol-displaceable component	17 ± 2 (61%)	0·2 ± 0·05 (3%)	0·2 ± 0·15 (3%)
Residual binding	11 ± 2	6·5 ± 1	5·5 ± 1

The results are the means (± 1 s.e.m.) of at least 2 experiments carried out on different lymphocyte preparations, the values in each experiment being the mean of triplicate determinations.

Lysis of cells using hypotonic 5mM Tris-HCl buffer containing 5 mM sodium chloride, following incubation with [^{3}H]spiperone (4 nM) for 60 min and before filtration, caused a dramatic reduction in the haloperidol (10^{-5} M) displaceable component of [^{3}H]spiperone binding to lymphocyte preparations. No reduction occurred in identical samples to which was added isotonic 140 mM sucrose-5 mm Tris-HCl buffer containing 5 mM sodium chloride (Fig. 2). In contrast, non-displaceable binding was reduced by less than 20% following cell lysis.

Microscopic examination of cell suspensions using a haemocytometer chamber showed greater than 98% lysis of lymphocytes had occurred within 2 min in the hypotonic medium. In the isotonic medium no cell lysis was observed.

Inhibition of active uptake mechanisms

Pre-incubation of lymphocytes with the metabolic inhibitor iodoacetate (1 mM) for 30 min at 37 °C did not affect the haloperidol displaceable binding of [^{3}H]spiperone (4nM) to human lymphocytes (Table 3). Replacement of the sodium ion content of the incubation buffer with lithium chloride (120 mM) also did not alter the haloperidol-displaceable component of [^{3}H]spiperone (4 nM) to lymphocyte preparations.

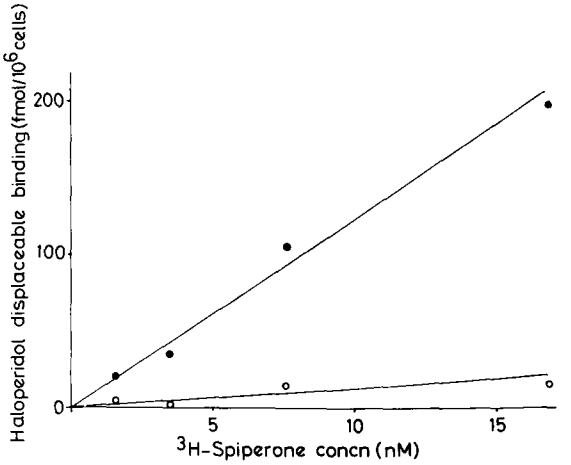

FIG. 2. The effect of cell lysis produced by a hypotonic Tris-HCl buffer on the haloperidol (10^{-5} M) -displaceable component of [^{3}H]spiperone (1·5–16 nM) binding to human lymphocyte preparation, compared with identical preparations treated with isotonic Tris-HCl buffer. Each value is the mean of two determinations carried out in triplicate at each ligand concentration and using separate lymphocyte preparations. Cells were incubated with [^{3}H]spiperone (with or without 10^{-5} M haloperidol) as described in the methods section. After 60 min incubation at 37 °C, 3 ml of either hypotonic or isotonic Tris-HCl buffer was added to each sample. After standing for 2 min samples were treated as described in the general methodology. Isotonic Tris-HCl buffer (sucrose 140mM, tris-HCl 5mM, NaCl 5mM, pH 7·4) Hypotonic Tris-HCl buffer (Tris-HCl 5mM, NaCl 5mM, pH 7·4).

DISCUSSION

We have carried out the experiments described in this paper in an attempt to reproduce the interesting findings of Le Fur & colleagues (1980; 1981a, b) that specific binding of [^{3}H]spiperone to dopamine receptors occurs in human lymphocyte preparations.

Initially we used the exact method described by Le Fur & colleagues. However, we found the results obtained difficult to interpret, because the binding of

Table 3. The effect of pre-incubation with iodoacetate (1 mM), or of replacement of sodium ions by lithium ions (120 mM), on the haloperidol (10^{-5} M) displaceable component of [^{3}H]spiperone (4 nM) binding to human lymphocyte preparations.

Addition	[^{3}H]spiperone bound (fmol/10^6 cells/nM)
None	23 ± 4
Iodoacetate	22 ± 3
None	18 ± 2
LiCl	18 ± 0·4

The results are the means (± 1 s.e.m.) of at least 2 experiments carried out on different lymphocyte preparations, the values in each experiment being the mean of triplicate determinations.

[^{3}H]spiperone to the Whatman GF/B filters employed (filter binding) was approximately equal to the apparent total binding of [^{3}H]spiperone occurring to lymphocytes (data not shown). Using smaller incubation volumes (200 µl) and employing GF/C filters designed for trapping cell suspensions, we have reduced filter binding of [^{3}H]spiperone to approximately 10% of the level of total binding occurring in the presence of lymphocyte preparations. Le Fur & colleagues also employed hypotonic lysis of red blood cells using Tris-HCl: NH_4Cl in the lymphocyte preparation. We examined this on a number of occasions and found it not to influence the binding of [^{3}H]spiperone (data not shown) so it was not employed subsequently. Lastly, although the number of cells we employed (10^6) was the same as originally described, the reduction in incubation volume employed raised the concentration of lymphocytes to 5 times that utilized by Le Fur & colleagues. It might be argued that a higher concentration of cells altered the binding characteristics of [^{3}H]spiperone. However, Le Fur & colleagues showed that for rat lymphocytes, the binding of [^{3}H]spiperone was linear between 10^4 and 2×10^6 cells ml^{-1}, although they did not go above this concentration. Extrapolation of their data, assuming the K_D of 4·8 nM and B_{max} value of 103 fmol/10^6 cells found by Le Fur & colleagues, shows our specific binding to human lymphocytes of 15–25 fmol/10^6 cells/nM [^{3}H]spiperone to compare well with a calculated value from their data of approximately 12 fmol/10^6 cells at 1 nM [^{3}H]spiperone. Additionally, there is good agreement between our study and that of Le Fur & colleagues as to the extent of specific binding. Using 10^{-5} M haloperidol to define displaceable binding, we achieved 60–75% displaceable binding compared with their value of 65–70%. Overall, therefore, the methodology we have employed does not appear to produce any major change in the characteristics of [^{3}H]spiperone binding to human lymphocytes.

With the present technique the characteristics of [^{3}H]spiperone binding to human lymphocytes do not confirm the existence of dopamine receptors on these cells as suggested by Le Fur & colleagues. We did not observe saturation of binding over a wide range of ligand concentrations, nor was there stereospecific displacement by the isomers of butaclamol. There was no correlation between the ability of the various drugs examined to displace [^{3}H]spiperone binding to human lymphocytes with their ability to displace this ligand from rat striatal preparations, or with their ability to act on dopamine, noradrenaline or 5-HT receptors. In addition, we examined two compounds structurally related to [^{3}H]spiperone which would displace it from a spirodecanone site. Such sites, which have no physiological significance have been found to exist in various areas of rat brain; they exhibit the characteristics of a saturable binding site from which displacement can only be obtained by close structural analogues of the ligand (Howlett et al 1979). However, these compounds did not show high affinity displacement of [^{3}H]spiperone binding to human lymphocytes.

Among the drugs studied, chloroquine was the most potent in displacing [^{3}H]spiperone from human lymphocytes. However, chloroquine possesses no selective dopaminergic activity, but in contrast is a lysosomotropic agent.

The failure to find specific saturable binding to membrane fragments suggests that a cell surface receptor is not involved in the association of [^{3}H]spiperone with lymphocytes. The fact that lysis of the cells at equilibrium caused a gross reduction in the amount of radioactivity associated with the lymphocytes suggests that the apparent binding of [^{3}H]spiperone is more likely due to intracellular accumulation of the ligand. This does not appear to be an active process since it was unaffected by the introduction of iodoacetate into the incubation medium, or by replacement of sodium ions by lithium ions in the incubation medium. It may represent simple passive diffusion of the ligand into the intracellular compartment of the cells from where it was displaced only by other drugs when incorporated in very high concentrations. If we assume that the lymphocyte volume is about 230 fl/cell, (Documenta Geigy 1970) and that [^{3}H]spiperone is evenly distributed within the cell, then, for 15 fmol of [^{3}H]spiperone to accumulate in 10^6 cells at a concentration of 1 nM in the medium, would require a concentration of approximately 75 nM [^{3}H]spiperone within the cell. This concentration gradient of 75:1 is of the same order of magnitude as the 25:1 concentration gradient that is achieved by platelets in taking up guanethidine or quinidine (Boullin & O'Brien 1968, 1971). Recently, Dulis & Wilson (1981) showed saturable uptake of [^{3}H]quinuclidinyl benzilate and [^{3}H]dihydroalaprenolol into polymorphonuclear leucocytes which could be displaced by related basic amines (IC50 values 10–10 000 nM), in particular chloroquine. This phenomenon appears to be due to uptake of basic amines into the acidic contents of lysosomes within polymorphonuclear leucocytes. On theoretical grounds Dulis & Wilson calculated that this pH-dependent mechanism

should result in approximately 500:1 concentration gradient across the lysosomal membrane. We would suggest, in view of the potency of chloroquine in our system, that we are observing a similar form of uptake process.

In conclusion, we cannot demonstrate the existence of dopamine receptors on the surface of human lymphocytes. This finding is in general agreement with those of other groups (Bloxham et al 1981; Maloteaux et al 1982). The apparent association of [^{3}H]spiperone with the lymphocytes would appear to be due to some passive uptake process into the cells. Le Fur & colleagues did show that binding of [^{3}H]spiperone to rat lymphocytes was consistent with the existence of dopamine receptor on the surface of these cells. However, they did not fully characterize the binding of the ligand to human cells and it may be that the existence of such receptors is species-dependent. Similar anomalies can be found in a species variation that occurs in the existence of receptors on the surface or erythrocytes. Thus, receptors are found on turkey erythrocytes (Levitzski et al 1974) which cannot be demonstrated on mammalian erythrocytes. Finally, whatever the process involved in the association of [^{3}H]spiperone with human lymphocytes, it might be of importance as an indicator of neurological and psychiatric disease. Using their methods, Le Fur & colleagues (1981a,b) have demonstrated that important changes occur in this process in Parkinson's disease. Further study is required to confirm this observation, and to assess the relevance of such changes to the disease process.

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REFERENCES

Andorn, A. C., Maguire, M. E. (1980) J. Neurochem. 35: 1105–1113

Bloxham, C. A., Cross, A. J., Crow, T. J., Owen, F. (1981) Br. J. Pharmacol. 74: 233P

Boullin, D. J., O'Brien, R. A. (1968) J. Pharm. Pharmacol. 20: 403–404

Boullin, D. J., O'Brien, R. A. (1971) Br. J. Pharmacol. 42: 114–126

Boyum, A. (1967) Scand. J. Clin. Lab. Invest. 97: (Suppl. 7) 77–89

Documenta Geigy (1970) 7th Edition p 618, J. R. Geigy, Basle

Dulis, B. H., Wilson, I. B. (1981) Biochem. Biophys. Acta. 643: 398–406

Fotino, M., Merson, E. J., Allen, F. H. (1971) Ann. Clin. Lab. Sci. 1: 131–133

Howlett, D. R., Morris, H., Nahorski, S. R. (1979) Mol. Pharmacol 15: 506–514

Le Fur, G., Phan, T., Uzan, A. (1980) Life Sci. 26: 1139–1148

Le Fur, G., Meininger, V., Phan, T., Gerard, A., Baulac M., Uzan, A. (1981a) Ibid. 27: 1587–1591

Le Fur, G., Meininger, V., Baulac, M., Phan, T., Uzan, A. (1981b) Rev. Neurol. (Paris) 137: 89–96

Levitzski, A., Atlas, D., Steer, M. L. (1974) Proc. Natl. Acad. Sci. U.S.A. 71: 2773–2776

Leysen, J. E., Gommeren, W., Laduron, P. M. (1978) Biochem. Pharmacol. 27: 307–316

Leysen, J. E., Awouters, F., Kennis, L., Laduron, P. M. Vandenberk, J. Janssen, P. A. J. (1981) Life Sci. 28: 1015–1023

Maloteaux, J. M., Waterkein, C., Laduron, P. M. (1982) Arch. Int. Pharmacodyn. Ther. in press.

Seeman, P. (1980) Pharmacol. Rev. 32: 229–313