Biochemical Pharmacology, Vol. 34, No. 7, pp. 941–947, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

EFFECT OF APOMORPHINE, α-METHYLPARATYROSINE, HALOPERIDOL AND RESERPINE ON DOPA PRODUCTION IN CLONAL CELL LINES (PC-12 AND N1E-115)*

M. BRÄUTIGAM†, G. LASCHINSKI, B. KITTNER and H. HERKEN
Institut für Pharmakologie, Freie Universität Berlin, Thielallee 69/73, D-1000 Berlin 33, Federal Republic of Germany

(*Received* 18 *May* 1984; *accepted* 11 *September* 1984)

Abstract—The effect of various drugs on DOPA production in the pheochromocytoma clone PC-12 and the neuroblastoma clone N1E-115 was studied. The N1E-115 cells contain only very low amounts of dopamine due to a lack of the aromatic L-amino acid decarboxylase, whereas the PC-12 cells are rich in dopamine. α-Methyl-*p*-tyrosine and apomorphine blocked DOPA production in both cell clones. Reserpine and haloperidol reduced the intracellular dopamine in the PC-12 cells and simultaneously induced a blockade of cellular DOPA production. The released dopamine was primarily recovered as 3,4-dihydroxyphenylacetic acid indicating a release of dopamine into the cytoplasm. This transient increase of cytoplasmic dopamine by reserpine or haloperidol brings about the inhibition of DOPA production in the PC-12 cells. Our results show that the PC-12 clone especially reacts to various drugs like other *in vitro* systems and may serve as an additional model for studying drug effects on catecholamine biosynthesis and metabolism.

The use of established clonal cell lines for pharmacological studies is widely accepted (for reviews see [1–4]. We have studied the effects of various dopaminergic agonists and antagonists on catecholamine biosynthesis in intact neuroblastoma N1E-115 cells [5, 6]. Due to a lack of the aromatic L-amino-acid-decarboxylase (AAAD)‡ [7], this clone contains only low levels of dopamine [5]. Greene and Tischler [8] established a rat adrenal pheochromocytoma cell line (PC-12) which contains AAAD and, thus, correspondingly high levels of dopamine.

The purpose of this study is to show that the effects of various drugs on catechol biosynthesis and metabolism—especially in the PC-12 clone—can be compared to the results obtained with established *in vitro* models, e.g. striatal slices and synaptosomes. In particular, the clonal lines represent a genetically stable and uniform cell population. Comparing the results obtained on PC-12 cells with those from the dopamine-lacking N1E-115 cells allows to distinguish indirect drug effects on DOPA production mediated via changes of intracellular feedback control by cytoplasmic dopamine.

* Dedicated to Professor Dr. med. Helmut Kewitz on the occasion of his 65th birthday. Parts of this investigation were supported by the Bundesministerium für Forschung und Technologie.

† To whom correspondence should be sent.

‡ Abbreviations used: AAAD, aromatic L-amino acid decarboxylase (EC 4.1.1.28); BH_4, (6*R*)-L-erythro-5,6,7,8-tetrahydrobiopterin; DA, dopamine; DOPA, 3,4-dihydroxyphenylalanine; DOPAC, 3,4-dihydroxyphenylacetic acid; DOPET, 3,4-dihydroxyphenylethanol; ELCD, electrochemical detection; NA, noradrenalin; NSD 1015, 3-hydroxybenzyl hydrazine; α-MPT, α-methyl-DL-*p*-tyrosine; PCA, perchloric acid; TH, tyrosine hydroxylase (EC 1.14.16.2).

MATERIALS AND METHODS

Cells and culture conditions

Pheochromocytoma clone PC-12 was a gift from Drs A. Sutter and A. Zimmerman (Berlin, F.R.G.), neuroblastoma clone N1E-115 was a gift from Prof. U. Littauer (Rehovot, Israel). Culture conditions were as described recently [9].

DOPA-production in intact PC-12 cells

Intracellular AAAD was blocked by 3-hydroxybenzylhydrazine (NSD 1015) and the resulting 3,4-dihydroxphenylalanine (DOPA) accumulation was measured. We used two models to assess DOPA-production in PC-12 cells.

(a) *Flask model.* NSD 1015 was added to the culture flasks (final concentration 5×10^{-4} M) 1 hr before the experiment. At the beginning of the experiment the cell monolayers were washed twice with warm medium, after which 20 ml of fresh culture medium (containing NSD 1015) were added. An aliquot of 500 μl medium was removed every 20 min to determine the DOPA content. The drugs were added after 60 min. After 140 min, the cell monolayers were washed with ice-cold 0.9% NaCl, harvested in 800 μl 0.2 M perchloric acid (PCA) with 10 mM dithioerytritol (to protect tetrahydrobiopterin (BH_4) from oxidation), sonicated, centrifuged at 15000 *g* (20 min) and assayed for intracellular BH_4, DOPA, dopamine (DA), noradrenalin (NA), tyrosine and cellular protein [10]. All values, including the accumulation of DOPA in the medium, were related to mg protein.

To determine changes of intracellular BH_4, DOPA, DA and tyrosine during this experimental procedure, cells in parallel cultures were treated as described above but were harvested at 20, 60 and

120 min to determine the mentioned intracellular parameters.

(b) *Micro test tube model.* A second system for testing drug effects on DOPA-production in intact PC-12 cells was devised to allow a more convenient testing of a larger number of drugs. After incubation for 1 hr with NSD 1015, washing, and addition of 20 ml culture medium (as described above), the cells were removed from the culture flask. One ml of this cell/culture medium suspension was pipetted into micro test tubes (1.5 ml; Eppendorf, Hamburg, F.R.G.). Simultaneously, the drug or control solution (100 μl) was added to obtain the required concentration. After 1 hr the micro test tubes were centrifuged at 600 r.p.m. for 1 min. An aliquot (500 μl) of the cell-free culture medium was assayed for DOPA. After removing the remaining culture medium, 400 μl of 0.24 M PCA and 200 μl of internal standard (α-methyl-DOPA and adrenaline in 0.1 M PCA) were added. The cells were then sonicated, centrifuged and assayed for intracellular DOPA and DA and cellular protein. All values were related to mg protein.

DOPA-production in intact N1E-115 cells

Because of the AAAD deficiency, the N1E-115 cells release more than 95% of the produced DOPA into the culture medium, which can be used to estimate DOPA-production of the cells (for details of the method see [6]).

Tetrahydrobiopterin assay

BH_4 was always determined immediately following the centrifugation step by HPLC and electrochemical detection (ELCD) (oxidation potential +250 mV; for details see [9] and [11]).

Studies of DA-metabolites in PC-12 cells

For studying the metabolites in the PC-12 clone after treatment with reserpine or haloperidol, cells were incubated for 1 hr in Hanks' balanced salt solution containing 10 mM hydroxyethylpiperazine–ethane–sulfonic acid (HEPES) (Seromed, München, F.R.G.), pH 7.4, and reserpine (1×10^{-6} M) or haloperidol (1×10^{-6} M). This buffer was used to prevent a possible further degradation of released catecholamine-metabolites by the culture medium normally used.

Catechol assay

DOPA-content in the culture medium was assayed after a purification step with aluminum oxide. Intracellular DOPA, DA and NA could be determined directly. The amount of catechols was determined by HPLC and subsequent electrochemical detection (oxidation potential +800 mV) (for details see [6]). For the determination of 3,4-dihydroxyphenylacetic acid (DOPAC) and 3,4-dihydroxyphenylethanol (DOPET), the following mobile phase was used to achieve a better separation: citrate–phosphate buffer (13.3 mM citric acid and 6.6 mM Na_2HPO_4), pH 3.3, containing 60 μM EDTA and 3.8 mM 1-octanesulfonic acid monohydrate (Fluka, Neu-Ulm, F.R.G.) with 10% methanol. The oxidation potential was set to +800 mV. No purification step was necessary prior to determination. The identity of cellular DOPAC and DOPET was verified by coelution with commercially available DOPAC and DOPET (Serva, Heidelberg, F.R.G.). Due to high intracellular levels tyrosine could be detected directly by HPLC and ELCD (oxidation potential +1000 mV) [6].

Drugs used

(−)-Apomorphine hydrochloride was a gift of Woelm Pharma (Eschwege, F.R.G.). 3-Hydroxy-benzyl-hydrazine (NSD 1015) and α-methyl-DL-*p*-tyrosine (α-MPT) were obtained from Sigma Chemie GmbH (Taufkirchen, F.R.G.). Haloperidol was a gift from Janssen GmbH (Düsseldorf, F.R.G.). Reserpine was from Fluka AG (Buchs, Switzerland). Reserpine was dissolved in glacial acetic acid (128 mg/ml), and haloperidol was dissolved in 1% tartaric acid (3.2 mg/ml); further dilutions were done in 0.9% NaCl. Apomorphine was dissolved in 0.9% NaCl containing 0.1% sodium metabisulfite. All other drugs were dissolved in 0.9% NaCl.

RESULTS

Figures 1 (a–e) show the behaviour of the various parameters of tyrosine hydroxylation during the test period in the flask model. After renewal of the culture medium (t = 0), most of the DOPA produced was released into the medium (Fig. 1a, b). The blockade of AAAD with 5×10^{-4} M NSD 1015 caused an increase in the accumulation of DOPA and simultaneously prevented the intracellular increase of DA (Fig. 1c). Higher concentrations of NSD 1015 (5×10^{3} M) did not further increase the accumulation of DOPA. This indicates a complete block of AAAD by 5×10^{-4} M NSD 1015. Therefore, this concentration was employed throughout the experiments. Two other important parameters for tyrosine-hydroxylase (TH) activity, intracellular tyrosine and BH_4, were assayed during the experiment. They did not change significantly (Fig. 1d, e). Intracellular NA content (90 μg/mg prot.) was always much lower than that of DA.

To test the stability of DOPA in the medium, a high concentration of α-MPT (5×10^{-4} M) was added to block TH-activity completely. Figure 2 demonstrates an immediate cessation of DOPA production; the DOPA concentration in the medium remained quite constant for the rest of the experiment. The intracellular parameters (DA, H_4B) with the exception of DOPA were not influenced by α-MPT.

Figure 2 demonstrates the effect of 2×10^{-6} M apomorphine in this test model. Apomorphine led to an almost complete blockade of DOPA production within 20 min. Again, the intracellular parameters (D4, H_4B), except DOPA, do not differ in apomorphine-treated flasks from controls.

A further simplification of the procedure described above (micro test tube procedure, see Materials and Methods) was used to examine drug effects in PC-12 cells. To compare both models, 5×10^{-4} M α-MPT was examined in this system. Figure 3 shows the course of total DOPA produced (intracellular DOPA + medium DOPA) and of intracellular DA content at 5, 20 and 60 min. DOPA production

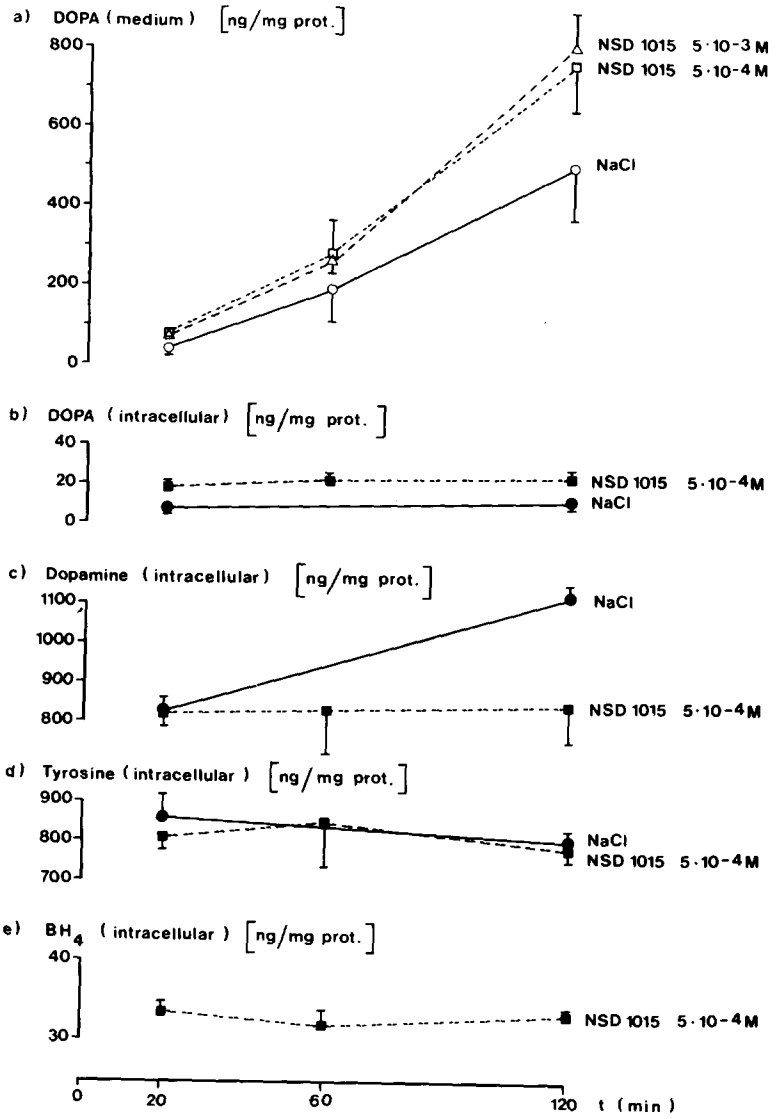

Fig. 1. Behaviour of various parameters of tyrosine hydroxylation after NSD 1015 during the test period in PC-12 cells (flask model): (a) accumulation of DOPA in the culture medium; (b) intracellular DOPA-accumulation; (c) intracellular DA-content; (d) intracellular tyrosine content; (e) intracellular BH_4 content. Each value represents the mean of three determinations ± standard deviation.

ceases almost immediately after addition of α-MPT, while, similar to the flask model, intracellular DA is not affected.

This system was used to test the effects of various drugs on DOPA production and on the storage of intracellular DA in PC-12 cells. Incubation with α-MPT and apomorphine blocked DOPA-production without affecting the intracellular storage of DA. Reserpine and haloperidol caused a significant loss of total intracellular DA and a simultaneous inhibition of DOPA-production (Table 1). Table 2 shows the effect of the various drugs on DOPA-production in the neuroblastoma clone N1E-115. This clone contains only very low levels of DA (~ 1 ng/mg protein). α-MPT and apomorphine show approximately the same effect in this clone as in PC-12 cells, but reserpine and haloperidol were completely ineffective.

In a separate experiment, the effects of reserpine and haloperidol on the release of DA from intracellular storage were examined more closely (Fig. 4). Normally the PC-12 cells contain only low levels of DA-metabolites (only DOPAC could be detected intracellularly). After 1 hr of incubation with reserpine or haloperidol, the total intracellular DA decreased sharply and most of the lost DA could be recovered extracellularly as DOPAC.

DISCUSSION

In the flask model (Fig. 1), almost all of the DOPA produced is released into the culture medium, if

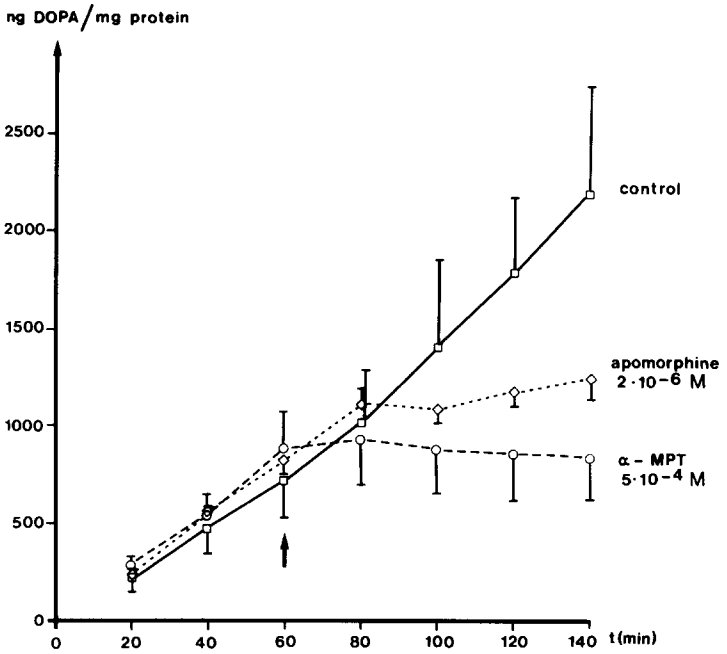

Fig. 2. Effect of 5×10^{-4} M α-MPT and 2×10^{-6} M apomorphine on DOPA-accumulation in PC-12 cells. Drugs were added 1 hr after the start of the experiment. Each value represents the mean of three determinations ± standard deviation.

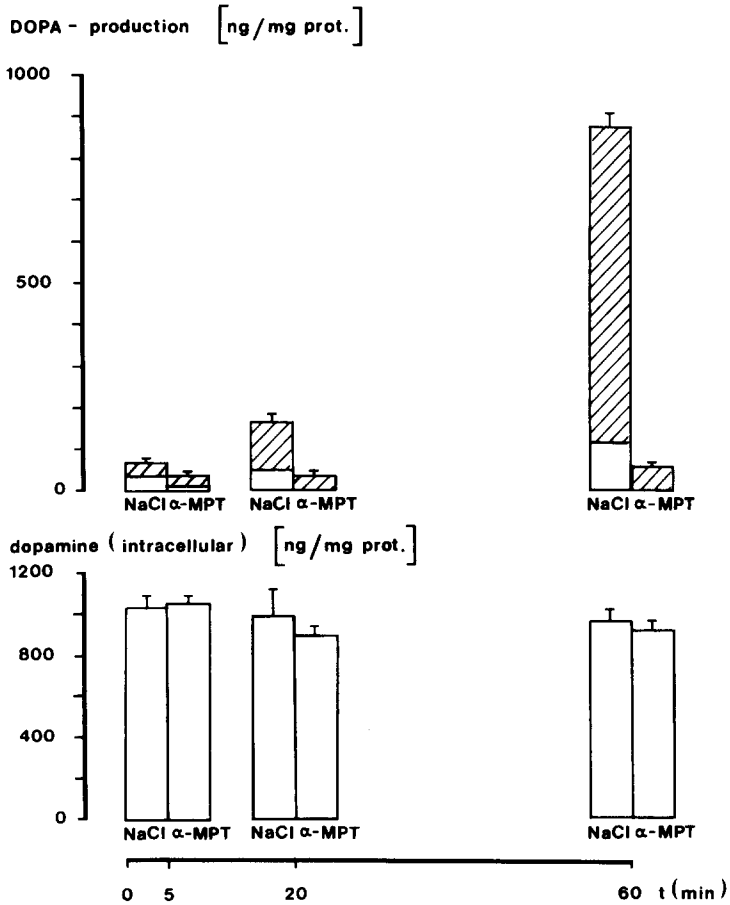

Fig. 3. DOPA-accumulation (intracellular and culture medium) and intracellular DA-content of PC-12 cells after 1 hr incubation with 5×10^{-4} M α-MPT, added at t = 0 (Micro test tube model) (the hatched part of the bars shows the amount of catechol found in the medium). Each value represents the mean of three determinations ± standard deviation.

Table 1. Effect of various drugs on (a) DOPA-production and (b) intracellular DA-content in PC-12 cells

Drug	Concentration of drug in culture medium (mole/l)						
	Controls	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
(a) DOPA-production in PC-12 cells (ng/mg prot. hr)							
α-MPT	1583 ± 355			1625 ± 17 (102.6)	1334 ± 114 (84.2)	443 ± 58 (28.0)	156 ± 18 (9.8)
Apomorphine	1579 ± 144	1445 ± 125 (91.5)	1417 ± 113 (89.7)	674 ± 38 (42.7)	209 ± 9 (13.2)		
Reserpine	2492 ± 61	1771 ± 66 (71.0)	1066 ± 65 (42.8)	1017 ± 30 (40.8)			
Haloperidol	1827 ± 132		1778 ± 73 (97.4)	1159 ± 112 (63.4)	852 ± 68 (46.7)	679 ± 19 (37.2)	
(b) DA-content in PC-12 cells (ng/mg prot.)							
α-MPT	1678 ± 32			1725 ± 26 (102.8)	1710 ± 48 (101.9)	1679 ± 18 (100.1)	1506 ± 6 (89.7)
Apomorphine	1494 ± 34	1449 ± 58 (96.9)	1404 ± 98 (94.0)	1472 ± 63 (98.5)	1394 ± 46 (93.3)		
Reserpine	1687 ± 3	1447 ± 64 (85.8)	922 ± 90 (54.6)	934 ± 50 (55.4)			
Haloperidol	1361 ± 48		1365 ± 53 (100.3)	1161 ± 56 (85.3)	874 ± 60 (64.2)	511 ± 57 (37.6)	

The cells were incubated with the drug during the whole test period (1 hr). The absolute values represent the arithmetic mean of three determinations ± standard deviation. The values in brackets indicate the percentage change as compared to the controls (= 100%).

AAAD is blocked by NSD 1015. Therefore, the DOPA content in the culture medium can be regarded as the total DOPA produced by the cells. The release of DOPA follows a concentration gradient and may be due to the relation of intracellular to extracellular (= culture medium) volume. In the experiment shown, e.g. in Fig. 1 (at 2 hr with 5×10^{-4} M NSD 1015), the DOPA concentration in the medium (20 ml) was 4×10^{-7} M. The corresponding intracellular DOPA was estimated to be 1×10^{-5} M (on the assumption that 1 mg protein corresponds to 10 μl intracellular water).

Considerable DOPA-release into the culture medium was also observed in the N1E-115 clone [6] and in suspensions of pheochromocytoma tumor cells [12, 13]. Ip *et al.* [14] have recently observed that considerable amounts of DOPA are also released by rat superior ganglia when they are explanted and examined in culture.

Compared to the N1E-115 clone, the PC-12 cells contain high levels of DA. Greene and coworkers (for review see [3]) have demonstrated vesicular storage of DA in the PC-12 cells. They also report a low NA content similar to our observation. Recently, Koike and Takashima [15] demonstrated clonal variability of the PC-12 cells with respect to their NA content.

Since the micro test tubes yielded basically the same results, both test systems were used for analysing pharmacological effects on tyrosine hydroxylation in intact cells. Our experiments show that various drugs are able to block DOPA production in PC-12 cells. As, however, different mechanisms appear to be responsible for this effect, each drug is discussed separately.

α-MPT

This drug is known to block TH by competition with tyrosine; Nagatsu *et al.* [16] and Udenfriend *et*

Table 2. Effect of various drugs on DOPA production in N1E-115 cells

Drug	Concentration of drug in culture medium (mole/l)						
	Controls	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
α-MPT	548 ± 112				504 ± 53 (92.0)	372 ± 31 (67.9)	106 ± 1 (19.3)
Apomorphine	418 ± 16	398 ± 48 (95.1)	326 ± 21 (78.1)	169 ± 48 (40.5)	57 ± 5 (13.7)		
Reserpine	564 ± 52	544 ± 30 (96.4)	537 ± 24 (95.1)	586 ± 55 (103.9)	584 ± 8 (103.4)		
Haloperidol	501 ± 83		573 ± 57 (114.4)	516 ± 18 (103.0)	507 ± 108 (101.0)	478 ± 33 (95.4)	

The cells were incubated with the drug during the whole test period (1 hr). The absolute values represent the arithmetic mean of three determinations ± standard deviation of cellular DOPA production (ng/mg prot. hr). The values in brackets indicate the percentage change as compared to the controls (= 100%).

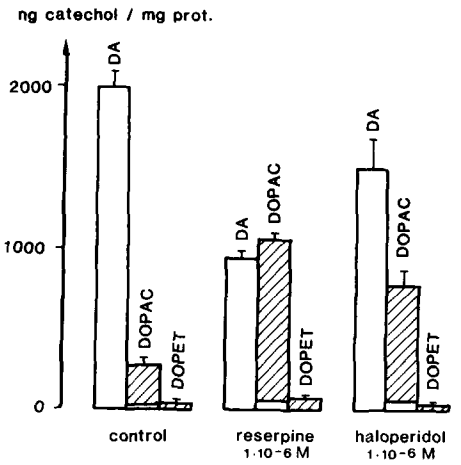

Fig. 4. Effect of 1×10^{-6} M reserpine and 1×10^{-6} haloperidol on DA and its metabolites in PC-12 cells during 1 hr incubation. DOPAC is the main metabolite after depletion of DA from its intracellular stores. Most of the produced DOPAC can be recovered in the medium (the hatched part of the bars shows the amount of catechol found in the medium). Each value represents the mean of three determinations ± standard deviation.

al. [17] have determined an IC_{50} around 2×10^{-5} M, which is compatible with our findings.

Apormorphine

The dopamine agonist apomorphine is widely used to study DOPA production in striatal slices and synaptosomes. The IC_{50} of apomorphine for blocking DOPA production in these models lies between 1×10^{-7} and 1×10^{-6} M [18–25]. In both the N1E-115 and PC-12 clones, production is inhibited by low concentrations of apomorphine ($IC_{50} < 1 \times 10^{-6}$ M). Neuroleptics were unable to reverse the apomorphine-induced blockade in the cell cultures. We recently showed that apomorphine can block TH activity in crude enzyme preparations obtained from rat striatum, N1E-115 and PC-12 clones at the same low concentration ($IC_{50} < 1 \times 10^{-6}$ M), provided the tests are performed at pH 7.2 and with BH_4 as cofactor [26]. We have, therefore, suggested that the inhibition of DOPA production by apomorphine is due mainly to a direct inhibition of TH. Since a partial reversal of the apomorphine inhibition by neuroleptics is observed in most studies on striatal slices and synaptosomes, an additional receptor-mediated effect on DOPA-synthesis may exist in these models. (For a more detailed discussion see [26].)

Reserpine

Reserpine is known to deplete DA from intracellular stores, mostly into the cytoplasm [27]. In our studies on the effect of reserpine on the DA-stores in the PC-12 cells we did not distinguish between cytoplasmic and vesicular DA. The significant augmentation of DA-metabolites after incubation with reserpine, however, indicates that the cytoplasmic DA must be increased; this is in accordance with the observed inhibition of DOPA production in PC-12 cells. In addition, reserpine had no effect in the DA-lacking N1E-115 cells.

Apparently most of the released DA is rapidly metabolized by the intracellular MAO to DOPAC, which readily leaves the cells. A concurrent blockade of DOPA-production shortly after treatment (40 min) with reserpine was also observed in rat striata *in vivo* [28], in striatal synaptosomes [29] and in striatal slices [22]. Interestingly, DOPAC seems to be the main DA-metabolite in *in vivo* studies as well [30].

Haloperidol

In vitro (synaptosomes) inhibition of DOPA-synthesis by neuroleptics has been described by several authors [19, 20]. Delanoy and Dunn [22] have recently suggested a reserpine-like effect of haloperidol in synaptosomes to explain the inhibition of DOPA production. This explanation tallies with our findings, especially as no blockade of DOPA production by either haloperidol or reserpine was observed [6] in the N1E-115 clone (containing only minimum amounts of DA). Moreover, the pattern of metabolites after haloperidol resembles that after reserpine. Seeman [31] pointed out that at aqueous concentrations of neuroleptics of more than 5×10^{-7} M, the neuroleptic concentration within the membrane can be very high. These high concentrations tend to fluidize all types of membranes, including vesicle membranes.

PC-12 cells can be used as a test model for studying the effects of drugs on DOPA-synthesis and metabolism in intact cells. These studies are certainly limited by the lack of neuronal activity of the cells, since it is known that the susceptibility of tyrosine hydroxylase to inhibition, e.g. by dopamine, can be altered by changes in impulse flow through dopaminergic neurons [32]. However, the PC-12 cell clone possesses a number of properties which makes a comparison to other *in vitro* systems (e.g. striatal slices and synaptosomes) possible. In contrast to these *in vitro* systems, the cell cultures represent a homogeneous material and important parameters of TH such as intracellular tyrosine, BH_4 and dopamine can be easily determined and thereby indirect drug effects may be elucidated. Due to its ability to synthesize and store DA, PC-12 cells may be useful for studying the mechanism of intracellular feedback control of catecholamine biosynthesis. In particular, the comparison with the N1E-115 cells, which contain little dopamine, may help to elucidate the role of this regulatory mechanism.

Acknowledgements—The authors wish to thank Mrs. Monika Bigalke, Miss Bettina Linke, Miss Petra Risse and Mrs. Evelyn Slania for their excellent technical assistance. We thank Dr. Schneider, Schering AG, Berlin, for his advice regarding the separation of the dopamine metabolites.

REFERENCES

1. X. O. Breakefield, *Life Sci.* **18**, 267 (1976).
2. E. Richelson, *Int. Rev. Biochem.* **26**, 81 (1979).
3. L. A. Greene, *Adv. Cell. Neurobiol.* **3**, 373 (1982).
4. H. Herken, *Klin. Wschr.* **61**, 1 (1983).

5. M. Bräutigam, R. Dreesen, C.-W. Flosbach and H. Herken, *Biochem. Pharmac.* **31**, 1279 (1982).
6. M. Bräutigam, R. Dreesen and H. Herken, *Naunyn-Schmiedeberg's Arch. Pharmac.* **320**, 85 (1982).
7. J. C. Waymire and K. Gilmer-Waymire, *J. Neurochem.* **31**, 693 (1978).
8. L. A. Greene and A. S. Tischler, *Proc. Natn. Acad. Sci. U.S.A.* **73**, 2424 (1976).
9. M. Bräutigam, R. Dreesen and H. Herken, *J. Neurochem.* **42**, 390 (1984).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
11. M. Bräutigam and R. Dreesen, *Hoppe-Seyler's Z. physiol. Chem.* **363**, 1203 (1982).
12. K. K. Vaccaro, B. T. Liang, B. A. Perelle and R. L. Perlman, *J. biol. Chem.* **255**, 6539 (1980).
13. R. E. Erny, M. W. Berezo and R. L. Perlman, *J. biol. Chem.* **256**, 1335 (1981).
14. N. Y. Ip, R. L. Perlman and R. E. Zigmond, *J. Pharmac. exp. Ther.* **223**, 280 (1982).
15. T. Koike and A. Takashima, *J. Neurochem.* **42**, 1472 (1984).
16. T. Nagatsu, M. Levitt and S. Udenfriend, *J. biol. Chem.* **239**, 2910 (1964).
17. S. Udenfriend, P. Zaltzman-Nirenberg and T. Nagatsu, *Biochem. Pharmac.* **14**, 837 (1965).
18. M. Goldstein, L. S. Freedman and T. Backstrom, *J. Pharm. Pharmac.* **22**, 715 (1970).
19. I. Christiansen and R. F. Squires, *J. Pharm. Pharmac.* **26**, 367 (1974).
20. L. L. Iversen, M. A. Rogawski and R. J. Miller, *Molec. Pharmac.* **12**, 251 (1976).
21. M. N. Bitran and G. Bustos, *Biochem. Pharmac.* **31**, 2851 (1982).
22. R. L. Delanoy and A. J. Dunn, *Biochem. Pharmac.* **31**, 3297 (1982).
23. B. A. McMillen, *Biochem. Pharmac.* **31**, 2643 (1982).
24. D. R. Haubrich and A. B. Pflueger, *Molec. Pharmac.* **21**, 114 (1982).
25. T. C. Westfall, L. Naes and C. Paul, *J. Pharmac. exp. Ther.* **224**, 199 (1983).
26. G. Laschinski, B. Kittner and M. Bräutigam, *Naunyn-Schmiedeberg's Arch. Pharmac.* **327**, 114 (1984).
27. R. Kuczenski, *Neuropharmacol.* **14**, 1 (1975).
28. A. Carlsson and M. Lindqvist, in *Neuropharmacology and Behaviour* (Eds. B. Haber and M. H. Aprison), p. 89. Plenum Press, New York (1978).
29. R. I. Patrick and J. D. Barchas, *J. Neurochem.* **23**, 7 (1974).
30. A. Zumstein, W. Karduck and K. Starke, *Naunyn-Schmiedeberg's Arch. Pharmac.* **316**, 205 (1981).
31. P. Seeman, *Pharmac. Rev.* **32**, 229 (1981).
32. V. H. Morgenroth, III., J. R. Walters and R. H. Roth, *Biochem. Pharmac.* **25**, 655 (1976).