PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 160, 344–348 (1979)

Effects of Psychotropic Drugs on DNA Synthesis in Cultured Lymphocytes (40447)

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The inhibitory effect of cannabinoids on cell division and macromolecular synthesis of cultured lymphocytes (1–3) and other eucaryotic (4, 5) cells has been reported by a number of investigators. It was suggested (6) that such an effect, which was observed with concentrations of 10^{-6}–$10^{-4}M$, is related in part to the liposolubility of these compounds in the double lipid layer of the plasma membrane (7). The purpose of the present study is to investigate if other commonly used psychotropic drugs produce a similar inhibition on cellular replication of cultured lymphocytes that might be related to their liposolubility. The drugs tested were: haloperidol (butyrophenone), chlorpromazine (phenothiazine), imipramine (dibenzazepine), diazepam, chlordiazepoxide (benzodiazepine), diphenylhydantoin, nialamide (MAO inhibitor), codeine (opiate), pentobarbital, phenobarbital, and meprobamate.

Material and methods. The technique used is that of Hartzman (8). Lymphocytes from venous blood of healthy male donors are collected by density gradient centrifugation with Ficoll Isopaque. Cells are suspended in RPMI 1640 containing 5% pooled human serum (a protein concentration that approximates that of extra cellular fluid), and 4 μg/ml of purified PHA (Burroughs Wellcome). Final concentration is 10^6 cells per milliliter. Lymphocytes are cultured for 72 hr and ^{3}H thymidine added at the 66th hr. Viability of cells is verified at the end of the culture with Trypan blue or phase contrast microscopy. Results are discarded when a higher than usual ratio (7.5%) of cellular death is found. In these series of experiments, cell count was not performed because of the clustering of cells by the mitogen. However, in another preparation, using spontaneously dividing L1210 murine lymphoma cells, cell count was performed in the presence of the same psychotropic drugs, and after 18 hr, a dose related decrease in number of cells related to the decrease in thymidine incorporation was observed. Drugs are solubilized in alcohol, mixed with the culture medium and added to the cultures so as to reach a final concentration of 5×10^{-7} to 10^{-3} M. Similar amounts of alcohol vehicle are added to the control cultures. As thymidine uptake varies considerably in different lymphocyte populations, uptake of the precursor into the test cultures is compared with its uptake into parallel control cultures. Results are then expressed in terms of percentage of precursor uptake in the control culture. The concentration of drug which inhibits 50% uptake of the precursor (I.C. 50) is obtained from the dose response curves. The I.C. 50 of these drugs is correlated with their octanol/water partition coefficient on log:log coordinates. Octanol/water partition coefficients used are those measured by Albert *et al.* (9) and by Seeman (10).

Results. Drugs tested produce an inhibition of thymidine uptake over a wide range of concentrations (Fig. 1). Haloperidol is the most powerful inhibitor (I.C. 50 = 10^{-5} M). However, concentrations as low as 5×10^{-7} M partially inhibit thymidine incorporation. Chlorpromazine has a similar I.C. 50 (1.3×10^{-5} M). Imipramine and diazepam display inhibitory effects close to that of THC (I.C. 50 = 4×10^{-5} and 7×10^{-5} M, respectively). Chlordiazepoxide (1.3×10^{-4} M) and diphenylhydantoin (1.1×10^{-4} M) have an intermediate cytotoxicity. Phenobarbital, nialamide, codeine, phenobarbital and meprobamate display a lower cytotoxicity with I.C. 50 of 5.5×10^{-4} M, 5.8×10^{-4} M, 9×10^{-4}, 1.2×10^{-3} M, 1.3×10^{-3} M, respectively. Ethanol has no inhibitory effects at a concentration as high as 10^{-1} M which corresponds to 5 g per liter. As represented on Fig. 2, the inhibitory effect of psychotropic drugs on thymidine incorporation is well correlated with their octanol/water partition coefficient ($r = 0.95$, $p < 0.001$).

Discussion. There is little data available on

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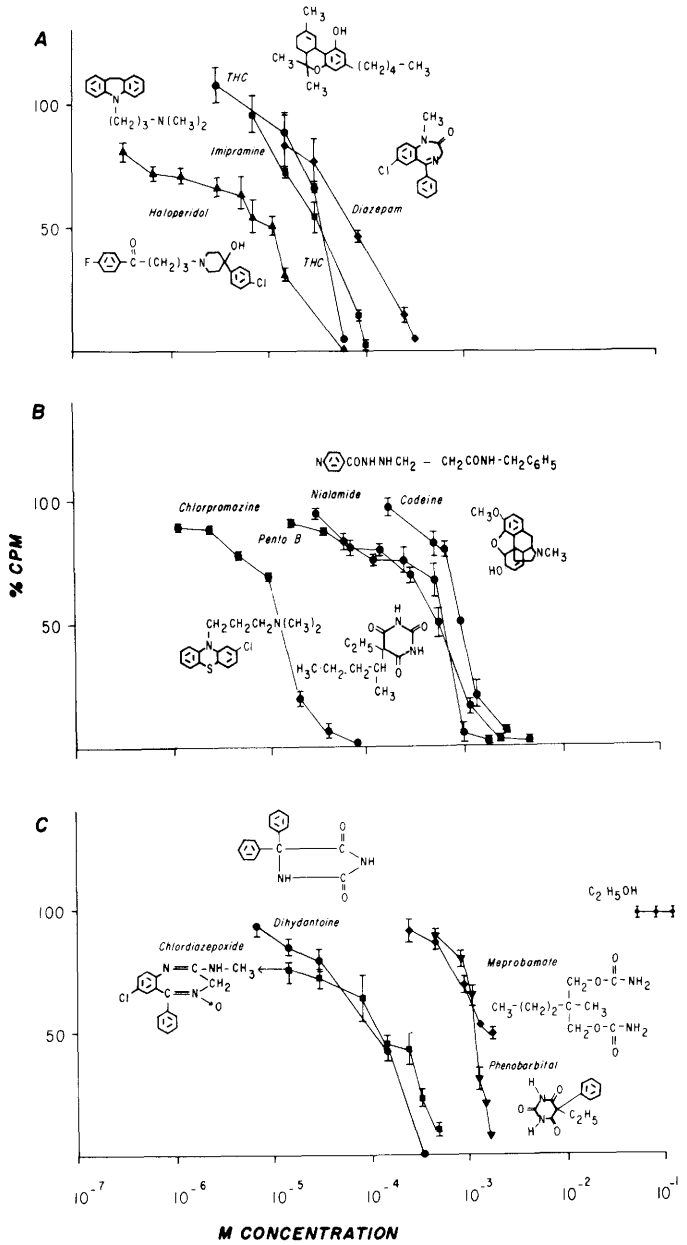

FIG. 1. Inhibitory effect of different psychotropic drugs on PHA induced lymphocyte transformation as measured by ^{3}H-thymidine incorporation after 3 days of culture. Inhibition of thymidine incorporation was calculated in reference to parallel control cultures. Each point represents the mean ± SE of four different experiments. A. Haloperidol, imipramine, tetrahydrocannabinol, diazepam. B. Chlorpromazine, pentobarbital, nialamide, codeine. C. Chlordiazepoxide, dihydantoin, meprobamate, phenobarbital.

the cytotoxicity of psychotropic drugs and on the effects of these drugs on lymphocyte blastogenesis. Ferguson et al reported (11) the inhibitory effects of chlorpromazine on Con A activated mouse spleen cells at a concentration of 5×10^{-6} *M*. Baker *et al.* (12) also reported the inhibitory effect of similar concentrations of phenothiazines and haloperidol on RNA synthesis in cultured human lymphocytes. These last authors postulated

that the observed inhibition was mediated through a specific receptor site. Others (13) have reported that diazepam 10^{-5} to 5×10^{-5} *M* inhibits protein synthesis in chicken embryo myoblasts and have concluded that the effect of this drug on muscle cell culture is "direct and specific".

This investigation indicates that the inhibitory effect of the different psychotropic drugs used is significantly correlated with their octanol water partition coefficient: the more lipophilic a substance is, the greater its inhibitory effect (Fig. 2).

Such a correlation might indicate that this

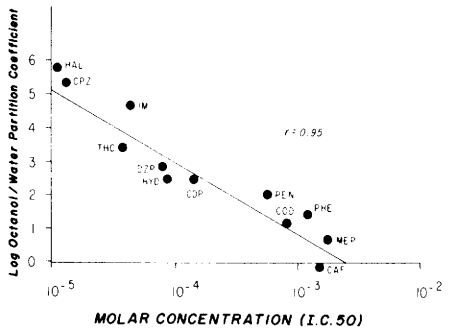

FIG. 2. The relationship between the molar concentration of different psychotropic drugs required to inhibit by 50% thymidine incorporation, and their octanol water partition coefficient (liposolubility). CPZ = chlorpromazine; DZP = diazepam; CDP = chlordiazepoxide; HAL = haloperidol; IMI = imipramine; HYD = hydantoin; PEN = pentobarbital; COD = codeine; PHE = phenobarbital; MEP = meprobamate; CAF = caffeine; THC = delta 9 tetrahydrocannabinol.

effect is exerted in part at the level of the double lipid layer of the membrane, and would be a "non specific" property of these lipophiles. This property was first described for anesthetics and tranquilizers by Seeman (10). This author studied the property displayed by these drugs to increase resistance of erythrocytes to 50% hemolysis (AH 50). The concentration of drug required to inhibit 50% incorporation of thymidine in the present experiments corresponds to that which protects against 50% hemolysis (AH 50) (Table I). Seeman has postulated that neuroleptics have a non specific anesthetic action related to their liposolubility in the membranes and to their property of expanding membrane proteins (14).

The resulting membrane area expansion will result in conformational changes of the phospholipid and protein components. As a consequence of membrane expansion, membrane bound enzymes may be inhibited (10, 15).

Such a "nonspecific" effect of these drugs is exerted with micromolar to millimolar concentrations which might be reached only in heavy, chronic consumption. By contrast, their specific psychotropic effects are exerted with nanomolar concentrations as a result of a stereoselective interaction with receptor sites located in the central nervous system (16).

Diphenylhydantoin (Table I) is the only one of all the drugs studied which in patients may reach a plasma concentration similar to the I.C. 50 observed in the present experi-

TABLE I. DAILY DOSE OF DIFFERENT PSYCHOTROPIC DRUGS, THEIR MAXIMAL MOLAR (M) PLASMA CONCENTRATION, AND THRESHOLD CONCENTRATIONS REQUIRED TO INHIBIT, *IN VITRO* THYMIDINE INCORPORATION INTO CULTURED LYMPHOCYTES.

	Daily dose (mg)	Max. Plasma Concentration (*M*)	"Threshold" IC (*M*)[a]	I.C. 50 (*M*)[b]	A.H. 50 (*M*)[c]	Log Octanol/water partition coefficient
Haloperidol	2–30	0.6×10^{-8} (32)	3×10^{-7}	1.1×10^{-5}	2.2×10^{-5}	5.74
Chlorpromazine	200–2000	2.0×10^{-6} (27)	5×10^{-6}	1.3×10^{-5}	1.0×10^{-5}	5.32
Imipramine	50–300	2.5×10^{-6} (33)	3×10^{-5}	4.3×10^{-5}	1.0×10^{-5}	4.62
Delta-9-THC	4–400	1.0×10^{-6} (35)	5×10^{-6}	3.0×10^{-5}	1.0×10^{-5}	3.20
Diazepam	5–30	3.5×10^{-6} (30)	4×10^{-5}	7.8×10^{-5}	4.0×10^{-5}	2.82
Chlordiazepoxide	20–160	6.0×10^{-6} (29)	4×10^{-5}	1.4×10^{-4}	3.0×10^{-4}	2.44
Hydantoin	100–500	9.0×10^{-5} (17)	1×10^{-5}	8.2×10^{-5}	1.5×10^{-3}	2.47
Phenobarbital	30–60	1.3×10^{-4} (17)	8×10^{-4}	1.2×10^{-3}	5.7×10^{-3}	1.42
Meprobamate	400–1200	0.8×10^{-4} (34)	5×10^{-4}	1.7×10^{-3}		0.70

[a] "Threshold" inhibiting concentration (producing significant inhibition of thymidine incorporation).
[b] Concentration of drug required to inhibit by 50% incorporation of ^{3}H thymidine.
[c] Concentration of drug to protect against 50% hemolysis (10).

ments (17). Chronic usage of this compound has been associated with clinical instances of immunological incompetence (18). The triggering mechanism of diphenyl–hydantoin induced myeloproliferative disease is suspected to be the inhibition exerted by this drug on folic acid metabolism (19). In view of the present findings, one should also take into account the inhibitory effect of this compound on macromolecular synthesis.

No such instances of clinical immunological incompetence have been reported with the usage of the other drugs studied in the present experiments. However, the use of phenothiazines and benzodiazepines during pregnancy has been associated by some investigators with abnormal fetal development (20–26).

With heavy chronic usage, chlorpromazine blood concentrations of 10^{-6} *M* have been reported (27). Since tissue concentrations may be 3–5 times higher, 2×10^{-5} *M* concentrations that inhibit *in vitro* precursor uptake and macromolecular synthesis may be reached in certain tissues. Furthermore, chlorpromazine is biotransformed into as many as 40 metabolites (28) which accumulate in tissues and which may have inhibitory properties on macromolecular synthesis similar to the parent compound. Unlike chlorpromazine, chlordiazepoxide (29) and diazepam (30) concentrations in plasma do not reach levels producing inhibition of macromolecular synthesis *in vitro*. However, these drugs give rise to active metabolites, such as desmethyldiazepam which has a higher plasmatic concentration than the parent compound and is also cumulative (30). When the plasma concentration of this metabolite is taken into account, a total plasma level of 10^{-5} *M* may be reached.

It is suggested that the inhibitory effect on DNA synthesis of these psychotropic drugs is related to their liposolubility and might be mediated in part through a common "non specific" mechanism on the plasma membrane which would decrease precursor transport. Evidence for such a mode of action has been described for the cannabinoids (31). However, other mechanisms might also be involved, and the effects of psychotropic drugs on all other macromolecular biosynthetic events of the cell require further investigations.

Summary. Commonly used psychotropic drugs in concentrations of 10^{-6}–10^{-4} *M* exert an inhibitory effect on DNA synthesis as measured by ^{3}H thymidine uptake into cultured lymphocytes. This effect is correlated with the octanol water partition coefficient (liposolubility) of these drugs ($p < 0.001$). It is suggested that this inhibition of macromolecular synthesis is a general effect exerted on all cells and requires micro to millimolar concentrations which may be reached only during chronic consumption. By contrast, the stereo selective effect of these compounds is exerted with nanomolar concentration on the receptor sites of biomembranes of the central nervous system.

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Received November 9, 1978. P.S.E.B.M. 1979, Vol. 160.