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Letter to the Editor

The Tricyclic Antidepressants Clomipramine and Citalopram Induce Apoptosis in Cultured Human Lymphocytes

ZHENLEI XIA*, JOSEPH W. DEPIERRE* AND LENNART NÄSSBERGER*†

*Unit for Biochemical Toxicology, Wallenberg Laboratory, University of Stockholm, 106 91 Stockholm and †Department of Medical Microbiology, University of Lund, Sölvegatan 23, 223 62 Lund, Sweden

Apoptosis, now referred to as programmed cell death (PCD), was first described by Kerr et al (1972). This phenomenon is important in the immune system and manifests itself through the process of internucleosomal DNA fragmentation by endogenous endonucleases. The mechanism of PCD is likely to be as important as mitosis in regulating growth and renewal of lymphocytes. Maturation of cells contributing to the complete immune network involves selective apoptosis during the positive and negative selection process.

Tricyclic antidepressants (TCA) are widely used for treatment of major and minor depressive disorders. It has been demonstrated that these compounds may affect cells related to the immune system (Lullmann et al 1975). We have found that the two TCAs clomipramine and citalopram induce apoptosis in lymphocytes. Mononuclear cells were isolated according to Bøyum (1968). Lymphocytes were separated from monocytes by applying the cell suspension to Costar wells and incubating for 1 h at 37°C. Thereafter, non-adherent cells were pipetted off and again incubated under the same conditions as mentioned above. This procedure was repeated four times to remove all monocytes and to obtain as pure a lymphocyte population as possible. The lymphocyte population was incubated up to 48 h with clomipramine (20 μM) and citalopram (160 μM), which were the lowest concentrations which induced clearly detectable apoptosis. In parallel experiments cells were pre-incubated for 1 h with aurintricarboxylic acid (ATA, 0·75 mM) or 3 h with cycloheximide (10–50 μM) before the respective TCA was added. Cells were collected and extracted with phenol/chloroform and the extract obtained was added to Eppendorf vials and spun down at 13 000 rev min^{-1} in a bench centrifuge.

The DNA extract was subjected to electrophoresis (1·5% agarose), where TCA-treated lymphocytes presented a typical DNA ladder pattern. Control cells and lymphocytes which had been pre-incubated with 0·75 mM ATA lacked the typical DNA ladder. In order to quantify the DNA fragments, cells were lysed and afterwards spun down at 27 000 g to separate small DNA fragments from intact DNA. The supernatant and the lysate were collected and stained with 100 ng mL^{-1} 4′,6-diamidino-2-phenylindole (DAPI) for 30–60 s, and the fluorescence intensity measured in these two subfractions at 365 and 454 nm, respectively, in a fluorescence spectrophometer. Table 1 shows the quantitative pattern (with DAPI staining) of the DNA fragments from nontreated cells, and cells incubated with clomipramine plus ATA, or with clomipramine alone. The protective effect of ATA is clearly illustrated. The explanation as to why clomipramine is 8-fold more potent in inducing apoptosis than citalopram is unknown. Pre-incubation with different concentrations of cycloheximide did not prevent lymphocytes from undergoing apoptosis when exposed to TCA.

TCAs have been reported to possess different effects on cells in the immune system. Lullman-Rauch demonstrated several years ago that TCAs induce lipidosis-like alterations in rat lymph nodes. Later on, citalopram was found to induce the same type of morphological alterations in peripheral lymphocytes (Lullmann-Rauch & Nässberger 1983). Recently it was shown that these compounds exert profound inhibition of the process of lymphocyte transformation (Mårtensson & Nässberger 1993). However, it is not clear whether the morphological findings described may be related to the increased frequency of apoptosis during incubation with TCA. Anyway, TCA-induced PCD in lymphocytes seems to be activated without the involvement of de-novo protein synthesis, since cycloheximide, an

Table 1. The quantitative profile of DNA fragments from human lymphocytes based on 4′,6-diamidino-2-phenylindole staining of supernatant and lysate, respectively. Lymphocytes were either incubated with clomipramine (20 μM) alone, or citalopram (160 μM) alone, or were first pre-incubated for 1 h with 0·75 mM aurintricarboxylic acid (ATA) before addition of the antidepressants. These incubations lasted for up to 48 h. Control cells were incubated with the vehicle alone.

	% DNA fragmentation	
	After 24 h	After 48 h
Control	12·6 ± 3·7	13·7 ± 2·0
Clomipramine 20 μM	31·0 ± 6·9*	41·5 ± 3·6***
Clomipramine 20 μM + ATA 0·75 mM	12·5 ± 2·4	13·5 ± 3·5
Citalopram 160 μM	23·5 ± 3·3*	36·0 ± 4·7**
Citalopram 160 μM + ATA 0·75 mM	14·2 ± 2·2	14·5 ± 2·5

* $P < 0·01$, ** $P < 0·05$, *** $P < 0·001$ compared with controls.

Correspondence: L. Nässberger, Unit for Biochemical Toxicology, Wallenberg Laboratory, University of Stockholm, 106 91 Stockholm, Sweden.

inhibitor of protein synthesis, was without effect. Increased PCD may prevent development of conditions like leukaemia, lymphomas, lymphoid hyperplasia or different autoimmune diseases, since resistance to PCD may be coupled to these disorders (Kroemer & Martinez 1994). In analogy, TCAs that induce apoptosis may therefore be useful in downregulating development of these disorders.

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Book Review

Psychopharmacology: the Fourth Generation of Progress
An Official Publication of the American College of Neuropsychopharmacology
Editors-in-Chief Floyd E. Bloom and David J. Kupfer
Published 1995 Raven Press, NY
xxxix+2002 pages
ISBN 0 7817 0166 X $220.50

This book represents the fourth in the series of official publications prepared for the American College of Neuropsychopharmacology on the state of the art in psychopharmacology. For those familiar with these editions, it is sufficient to say that the fourth issue, despite having a slightly different format, maintains the review style of presentation and is even heavier than before. Newcomers to the series must surely be newcomers to psychopharmacology but they can be certain that whatever their discipline, it will be represented, and wherever appropriate, the review will be an upgrade from the previous 'generation'.

The editors have subdivided the book simply along preclinical or clinical lines having much cross-reference, with a third small section headed Special Topics regarded by the Editors-in-Chief as topics of the future.

Each of the major sections is introduced by a chapter giving an overview of and a rationale for its contents. For the reviewer seeking to understand the aims of the editors, these chapters are quite useful though their main function appears to be a replacement for what might have been a rather long editorial. Following this single chapter each section considers, in turn, some of the pertinent methodology, the major target areas and a series of integrative concepts which cannot neatly be parcelled in with the main target areas.

The chapters on methods in both the preclinical and clinical divisions are successful additions, which should prove particularly useful for the new student or the supervisor approaching new disciplines. However, a chapter on statistical methodology would not have gone amiss, and although the chapter on neuropsychological testing is an excellent review of methods available to monitor dementia, there is surely an argument for a more general review of neuropsychological testing in the context of psychopharmacology.

The major body of the preclinical section focuses on neurotransmitters both traditional and those whose credentials are less well established. Where appropriate, the reviewers have taken the previous generation as the starting off point and where possible have proceeded along one of the four major alignments of neuroscience research — molecular, cellular, multicellular or behavioural — thus providing a wide-ranging database for the neurobiologist of almost any persuasion. The extent to which this has proved possible reflects the volume of research data available. For the newer neuromessengers such as arachidonic and nitric oxide, there are single chapters which will undoubtedly prove to be growth areas of the future.

The clinical section is presented similarly with specific disorders being the target areas, reviewed in relation to both psychobiology and treatment. Mood disorders and schizophrenia receive the largest input but the greatest increase in coverage, reflecting the advances made since the previous generation, is apparent in the geriatric disorders, the neurological disorders, AIDS and substance abuse. There is, inevitably, some degree of selectivity but this section should prove to be a valuable source book for the practising clinician as well as the research psychiatrist.

The themes discussed under Special Topics seem to represent areas of special interest for members of the pharmaceutical industry, but the chapters on ethical and cultural aspects of psychopharmacology and on violence and aggression highlight the omission of chapters specifically addressing pharmacogenetics and forensic psychopharmacology, respectively.

With such extensive cross-referencing between chapters, chapter numbers on the page headings would have been helpful. Otherwise, the book is well presented, well indexed and, on the whole, very readable, even if there is a tendency for the European and southern hemisphere literature to be poorly represented. Concern must be expressed, however, that the generations are becoming shorter with each issue and at the present rate of progress, within the next 30 years, there will be 'annual' generation — and that may be more than most bookshelves can support.

E. F. MARSHALL
UNIVERSITY OF NEWCASTLE UPON TYNE, UK