Effect of marsilid on dopamine(DA)-effect

Treatment	Cell type	Growth of cell population Inhibition (%)	Stimulation (%)
DA (50 µg/ml)	NB	56.0 ± 6.0	
DA (50 µg/ml) + marsilid	NB	—	10.0 ± 8.0[a]
DA (100 µg/ml)	NB	71.0 ± 4.0	—
DA (100 µg/ml) + marsilid	NB	2.0 ± 5.0	—
Marsilid	NB	—	21.0 ± 4.0
Marsilid	BHK-21	—	27.0 ± 4.0

[a] Standard deviations. Marsilid (100 µg/ml) was added 24 h after plating. After 18–20 h of incubation in the presence of marsilid, dopamine (50 and 100 µg/ml) was added and then incubated for 1 h. After incubation cells were washed twice with F12 medium without serum and fresh growth medium was added. The total number of cells was counted 2 days after dopamine-treatment. The experiment was repeated thrice and each value represented an average of at least 6 samples.

washed twice and fresh growth medium was added. The cell number in drug-treated and control neuroblastoma culture was compared 3 days later.

Results and discussion. The Table shows that marsilid completely prevented the dopamine-induced inhibition of cell division. Dihydroxyphenylacetic acid (DPAA) an acid metabolites of dopamine did not effect the growth of neuroblastoma cells. MAO converts 3,-methoxytyramine, an intermediate metabolite of dopamine, to homovanillic acid; however, homovanillic in the previous study[1] produced no effect on the growth. These results indicate that amine-dervived aldehyde may be responsible for the dopamine-effect. This study also shows that marsilid stimulated the growth of neuroblastoma as well as of Baby-hamster kidney cells in vitro (Table), indicating that this marsilid-effect is not specific for neuroblastoma cells. The growth medium in the presence of pyrogyllol (100 µg/ml) turned dark-brown within 1 h after treatment, indicating the auto-oxidation of the compound. Addition of ascorbic acid (20 µg/ml) did not prevent the auto-oxidation. The COMT inhibitor under above experimental condition reduced the growth of neuroblastoma cells to about 6% of controls. The mechanism of marsilid-induced stimulation of cell division is unknown. However, it may be postulated that cellular-aldehyde may be one of the important factors in controlling the cell division.

Zusammenfassung. Die hemmende Wirkung von Dopamin auf die Zellteilung von Neuroblastoma beruht wahrscheinlich nicht auf dem Effekt seines desaminierten Metaboliten, obwohl sie durch den MAO-Hemmer Marsilid aufgehoben wird.

K. N. PRASAD[5]

Department of Radiology University of Colorado Medical Center, 4200 East Ninth Avenue, Denver (Colorado 80220, USA), 28 May 1971.

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Effect of Catatoxic Steroids upon Established Morbid Changes

A considerable amount of evidence has accumulated during recent years in support of the concept that catatoxic steroids exert most of their protective effects by destroying toxicants (e.g., through the induction of hepatic microsomal or other enzymes). Thus, pretreatment with spironolactone, ethylestrenol, norbolethone and many steroid carbonitriles has been shown to offer protection against the subsequent administration of digitoxin[1], mercury[2], pesticides[3], carcinogens[4], nicotine[5], excessive amounts of steroid hormones[6,7], tyrosine[8], and well over 100 other toxicants, widely differing in their chemical structure and pharmacologic actions[9]. These findings suggest interesting clinical applications in the prevention of diseases caused by exogenous or endogenous intoxications.

Defensive enzyme induction by these steroids may be more or less rapid but usually several days of pretreatment are necessary to obtain optimal protection. Some catatoxic effects do develop more rapidly, but of course immediate improvement of intoxications cannot be expected from substances which act through the time-taking process of defensive enzyme synthesis and do not neutralize toxicants directly, nor block their damaging actions at the level of the target organs.

In view of these considerations, the clinical applicability of catatoxic steroids appeared to be limited to the prophylaxis of predictable intoxications, such as unavoidable transient occupational exposure to pesticides, carcinogens, mercurials or other chemicals amenable to this type of detoxication. It was thought that by the time spontaneous diseases (caused by endogenous toxicants or accidental poisoning with exogenous materials) would produce symptoms permitting a diagnosis, the catatoxic steroids could no longer be of avail since they do not affect the established lesions themselves.

To check this assumption, 2 experiments were performed: the first to determine the duration of catatoxic steroid pretreatment required to obtain protection; the second to verify whether an already manifest intoxication could be cured by merely blocking the continued pathogenic action of the causative agent.

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We used exclusively female ARS/Sprague-Dawley rats with a mean body weight of 100 g (90–110 g) kept on Purina Laboratory Chow. In the first experiment, digitoxin (1 mg in 1 ml water, p.o., once on the first and 2 mg/day from the 2nd day to the termination of the experiment on the 7th day) was administered to all groups including the controls. Spironolactone (10 mg in 1 ml water, p.o., twice daily) was given beginning at the times indicated in the Table, concurrently or before initiation of digitoxin treatment. The intensity of the characteristic digitoxin convulsions was appraised in terms 0 to 3 plus on the 3rd day and the statistical significance of the apparent differences between the treated and control animals was computed by the 'Exact Probability Test' of FISHER and YATES as described elsewhere[9].

It is evident from the Table that even if digitoxin and spironolactone treatment is simultaneously initiated, the latter compound does offer some protection against and LD_{100} of digitoxin but only by delaying the onset and severity of the convulsions (as measured on the 3rd day). Eventually, all animals so treated died in severe convulsions. However, under otherwise identical conditions, 24 h of pretreatment with spironolactone sufficed to diminish mortality from 100% to 30%. Pretreatment for 48 h or more prevented all signs of intoxication.

The second experiment was performed with pregnenolone-16α-carbonitrile or PCN (IUPAC designation: 3β-Hydroxy-20-oxo-5-pregnene-16α-carbonitrile, SSS designation: $P^5CN_{16\alpha}ol_{3\beta}on_{20}$) which proved to be the most potent among over 500 steroids tested for catatoxic activity up to now[9]. The techniques were essentially the same as in the first experiment but the rats were chronically poisoned with digitoxin (0.5 mg on the first 4 days and 1.0 mg p.o./day thereafter) or indomethacin (0.75 mg s.c./day). With indomethacin the administration of PCN (1 mg × 2/day p.o.) was started on the 5th day, when clinical signs of peritonitis became evident. In the case of digitoxin, convulsions appeared 24 h after the first injection of the toxicant and PCN treatment was initiated at that time at the dose of 100 μg raising this amount to 1 mg × 2/day p.o. by the 5th day. The experiment was terminated on the 13th day. In both these experiments PCN caused the manifestations of intoxication to disappear and permitted survival despite continued daily treatment with the toxicants. Under identical conditions the controls receiving no PCN invariably died: digitoxin causing convulsions, indomethacin, characteristic intestinal ulcers (cf. Figure 1).

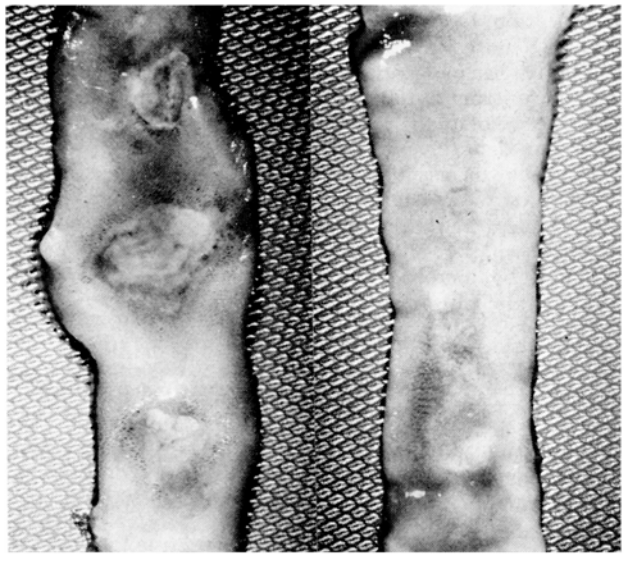
It is generally agreed that the protective effect of PCN against digitoxin and indomethacin depends upon the induction of drug-metabolizing enzymes[9]; yet the present observations show that – if the intoxication is sufficiently slow to permit effective enzyme induction in time – the steroid can act, not only prophylactically, but also curatively.

Of course, in the case of very acute and transient intoxications (e.g., steroid anesthesia, zoxazolamine paralysis) or rapidly fatal poisonings (e.g., with strychnine or picrotoxin) catatoxic steroids manifest only prophylactic effects[9]; however, their protective action can reach a very high level within 2 days. This point is important in appraising the possible value of catatoxic steroid therapy in spontaneous diseases which cannot be treated before they have produced detectable signs and symptoms[10].

Influence of the duration of spironolactone petreatment upon its antidigitoxin effect

Spironolactone (beginning h before digitoxin)[a]	Convulsions (scale: 0–3)	Mortality (dead/total)
Controls	3.0	10/10
0	1.4 ± 0.44[b]	10/10
24	0[c]	3/10
48	0[c]	0/10
72	0[c]	0/10
96	0[c]	0/10

[a] In addition the animals of all groups received digitoxin as explained in the text. [b] $P < 0.01$, [c] $P < 0.001$.



Mucosal surfaces of the small intestines of two rats given indomethacin. Left: 3 large ulcers in an animal which received no other treatment. Right: No ulcers in an animal which was given PCN only after manifestations of peritonitis had become evident.

Résumé. Chez les rats, une résistance à la digitoxine et à l'indométhacine peut être rapidement induite par des stéroïdes catatoxiques tel que la spironolactone ou la pregnénolone-16α-carbonitrilée (PCN). En fait, la PCN peut encore sauver la vie de l'animal en dépit d'un traitement utilisant des doses normalement mortelles de digitoxine ou d'indométhacine, même si le traitement par ces stéroïdes n'est commencé qu'après que des signes d'intoxication sont déjà devenus manifestes. Evidemment, les stéroïdes catatoxiques ne sont pas seulement des agents prophylactiques, ils peuvent également guérir des lésions morbides déjà établies.

H. SELYE

Institut de médecine et de chirurgie expérimentales, University of Montreal, C.p. 6128 Montreal (Québec, Canada

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