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EFFECT OF DOTHIEPIN ON NOCICEPTIVE RESPONSE IN DIABETIC RATS

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Abstract : In alloxan-diabetic rats of 4 wk duration with blood glucose levels of about 300 mg/100 ml, the tail flick reaction time (TFRT) to thermal stimuli was significantly elevated ($P<0.25$), indicating hypoalgesia.

Intraperitoneal dothiepin, injections of 25 mg & 50 mg/kg body weight per day did not significantly alter the TFRT, either in control or in diabetic rats, following either acute (one dose), or short term (once a day for five days) administration. It is concluded that at least in the dosage schedule used herein, dothiepin does not influence hypoalgesia of diabetic neuropathy.

Key words : alloxan-diabetes, hypoalgesia, tail-flick response, antidepressants, dothiepin

INTRODUCTION

Diabetes mellitus, a major health problem, produces neuropathy as the commonest complication (1). This has autonomic and/or peripheral neuronal components. Peripheral neuropathy may be either painful (hyperalgesia syndromes), or painless (numb extremities). Paradoxically, both types may be present in the same patient (2).

Diabetic neuropathy is associated with decrease in nerve conduction velocity (NCV) (1) which in the laboratory animals has been reported to occur after more than 2 wk (3) or 3 wk (4) following the induction of diabetes.

Tricyclic antidepressants (TCA) are commonly used in diabetic (hyperalgesic) neuropathy to give relief from pain (1, 2). There is no information however, on the use or efficacy of TCA in diabetic hypoalgesia.

Diabetic hyperalgesia responds to TCA via serotonergic, opioid neuropeptidergic and/or indirect antidepressant mechanisms (5). Diabetic hyperalgesia may attenuate as a function of antidepressant response. It is speculated that diabetic hypoalgesia may also attenuate similarly.

The aim of the present study was to assess the effect of dothiepin on thermal nociceptive responses in experimental diabetes induced by alloxan.

METHODS

Adult albino Wistar rats (140-230 g) of both sexes, housed at an ambient temperature of 27.03 ±2.41°C, were fed on standard pellet chow (Hind Lever) and tap water through drinking bottles. They were randomized into two groups and were given either distilled water (DW) or freshly prepared alloxan (70 mg/kg) through the caudal vein. After 24 hr, glycosuria was confirmed. Blood glucose was tested in the 4th week by the glucose oxidase method using Ames Glucometer Model No. 5580. Only diabetic rats with blood glucose values of more than 300 mg/100 ml were used for the study.

Using the Techno Analgesiometer, tail flick reaction time (TFRT) was noted before and at the end

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of the 1st, 3rd, and 4th week after injection with alloxan or DW. To prevent heat induced damage and scarring of the tail, the maximum cut off time allowed on the analgesiometer was 6 seconds. Only those rats having an initial TFRT less than 4 seconds were included in the study.

After completion of 4 wk of diabetes, both control and diabetic rats were randomly divided into 3 groups (n=6 per group) and were treated either with DW, or freshly prepared dothiepin in DW in a dose of 25 or 50 mg/kg, given ip at 9.00 am each day for 5 consecutive days. The TFRT was noted before, 2 hr after the first dose (which result indicated acute effect) and 2, 5, 24, 72 hr after the 5th dose of dothiepin (which result indicated delayed effect).

Statistical analysis of TFRT in control and diabetic rats was done by 2 way repeat measures ANOVA, while the data on TFRT before and after dothiepin were analysed by 3 way repeat measures ANOVA. Statistical significance was set at $P<0.05$.

RESULTS

The TFRT of 18 diabetic and 18 control rats was assessed before and 1, 3 and 4 weeks after induction of diabetes. Table I shows that the diabetic animals had a significantly ($F = 6.932$, $df = 1, 34$, $P<0.025$) elevated TFRT, indicating development of hypoalgesia.

TABLE I : Tail-flick reaction time in control and diabetic rats.

Group	*Pre-vehicle/ Alloxan*	*Post-vehicle/ Alloxan*		
		1 wk	*3 wk*	*4 wk*
Control	3.04±0.46	3.29±0.77	3.42±0.88	3.48±1.01
Diabetic	3.23±0.54	3.76±0.93	4.23±1.36	4.15±1.37

n = 18 in each cell, Values are Meant ± SD.
Inference of the two way repeat measures ANOVA are presented in the Results section.

No gross behavioral changes were observed in rats treated with dothiepin, when compared with DW treated rats. The data on TFRT before and after a single dose (acute) administration of dothiepin i.p. are presented in Table II. These data were analysed using 3 way repeat measures ANOVA, the variables being group (control and diabetic), treatment (DW, dothiepin 25 mg and 50 mg per kg), and time (predrug and 2 hr post drug). The main effect for group approached significance ($F=3.463$, $df=1, 30$, $p=0.08$), indicating that diabetic animals had higher TFRT than controls irrespective of treatment and time of assessment, supporting the earlier results that diabetic animals exhibited hypoalgesia.

TABLE II :Tail-flick reaction time in control and diabetic rats 2 hr after single dose of DW/dothiepin 25 or 50 mg/kg, i.p.

Group	*Treatment mg/kg*	*Pre-drug*	*Post-drug*
Control	DW	3.78 ± 1.10	4.09 ± 1.32
	Dothiepin 25	3.61 ± 1.29	3.47 ± 1.28
	Dothiepin 50	3.05 ± 0.51	3.03 ± 0.54
Diabetic	DW	4.45 ± 1.56	5.20 ± 0.94
	Dothiepin 25	4.58 ± 1.56	3.65 ± 1.09
	Dothiepin 50	3.42 ± 0.63	2.94 ± 0.58

n=6 in each cell, values are Meant ± SD.
Inferences of the 3 way repeat measures ANOVA are presented in the Results section.

The treatment x time interaction was nonsignificant ($F=2.134$, $df=2.30$, N.S.), as also the group x treatment x time interaction ($F=0.719$, $df=2,30$, N.S.). This indicated that the treatment categories changed comparably across time, and that this observation of comparable change remained true even when the treatment categories were considered separately in diabetic and control groups.

No other inference drawn from the ANOVA (main effect for time, main effect for treatment, group x time interaction, group x treatment interaction) emerged significant. As these were not germane to the objectives of this study, these inferences are not considered further.

The TFRT at various time intervals after the last of 5 daily (short term) i.p. injections of DW and 25 and 50 mg/kg of dothiepin, in control and diabetic rats respectively are presented in Table III. The results were analysed by 3 way repeat measures ANOVA as

TABLE III : Tail flick reaction time in control and diabetic rats after DW/dothiepin 25 or 50 mg/kg, ip once daily for 5 days.

Group	*Treatment*	*Pre-drug*	*Time in hours after the last dose of dothiepin*			
			2	5	24	72
Control	DW	3.78± 1.10	3.85± 1.24	3.79± 1.38	4.14± 1.58	3.55± 1.40
	doth. 25	3.61± 1.29	3.45± 1.12	3.24± 0.79	3.86± 1.03	4.12± 0.69
	doth. 50	3.05± 0.51	3.29± 0.92	2.72± 0.49	3.08± 0.60	2.89± 0.68
Diabetic	DW	4.45± 1.56	4.81± 1.17	5.08± 1.29	4.90± 1.28	5.41± 0.94
	doth. 25	4.58± 1.42	3.73± 0.81	3.58± 1.28	3.99± 1.02	3.94± 1.19
	doth. 50	3.42± 0.63	3.56± 1.50	2.83± 0.70	3.19± 0.80	2.97± 0.51

n=6 in each cell, values are Mean±SD.
Inferences of the 3 way repeat measures ANOVA are presented in the Results section.

before. There was a significant main effect for group ($F=4.77$, $df=1, 30$, $P<0.05$), indicating that diabetic rats continued to have a higher TFRT (hypoalgesia) when compared with controls.

Treatment x time interaction and group x treatment x time interaction were again nonsignificant ($F=0.97$, $df=8, 120$, N.S. and $F=0.70$, $df=8,120$, N.S. respectively), indicating that treatment did not influence TFRT scores at serial assessments even when considered separately as a function of group to which the rats belonged.

Other inferences drawn from this ANOVAwere not significant and, as before, not considered further.

DISCUSSION

The significant increase in spontaneous pain threshold to thermal stimuli in diabetic rats indicated development of hypoalgesia due to diabetes. This is in agreement with the results of Chu et al (6) who reported an increase in pain threshold after 2 weeks of experimental diabetes. Forman et al (7) reported a decrease in pain threshold alongwith a decrease in central and peripheral endogenous opioid levels. The difference in observations cannot be accounted for satisfactorily, but may be due to differences in the duration of diabetes.

The analgesia observed may be due to a decrease in conduction in peripheral nerves (3), or spinal cord (8), or due to alterations in the levels of endogenous opioid peptides in the anterior pituitary or hypothalamus (7), or may be due to an interplay of several factors.

Having established diabetic hypoalgesia, it seemed reasonable to assess whether TCA produces alterations therein, as TCA attenuates diabetic hyperalgesia via indirect intidepressant mechanisms. It may be speculated that diabetes produces cognitive-emotional changes which influence perception of stimuli for example apathy, lethargy or depressive equivalents may induce a functional exaggeration of diabetic hypoalgesia. It may be further speculated that TCA, by reversing such cognitive-emotional states, may attenuate diabetic hypoalgesia. This hypothesis has not been tested to date.

The results of this preliminary study indicate that neither acute, nor short term treatment with dothiepin, decreases diabetic hypoalgesia. One possible explanation for this negative finding may be that 5 days is too short a period for therapeutic effects of dothiepin to emerge, despite evidence suggesting that attenuation of diabetic hyperalgesia occurs in such a short term (5). Further studies are warranted to assess the influence of chronic TCA on diabetic hypoalgesia.

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