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Evoked Potentials in Diabetic Syndrome of Rats Before and After Two Months of Methadone Treatment

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The effect of two months treatment with methadone (0.87 mg/kg per os, daily) on the somatosensory evoked potentials (SEPs) in female control and diabetic rats were studied. Diabetes was induced by alloxan monohydrate (65 mg/kg *i.v.*). SEPs obtained after electrical stimulation of the contralateral forepaw and recorded from the scalp, by an non invasive method, showed high reproducibility. Prolonged latency (156%) and a marked amplitude enhancement (371%) characterized the mid-latency SEPs following 64 days methadone treatment of healthy rats. Experimental diabetes induced significant alteration of both parameters; amplitude decrease of earlier components (N1, P2) and delayed latency of later ones (P3). Two months methadone treatment of diabetic rats not only leads to the recovery but also augmented P2 (P25) amplitude (225%). It was concluded that no essential differences exist in the methadone effect on control (healthy) and diabetic Wistar female rats.

INTRODUCTION

Methadone (amidone; Figure 1) has the structural features common to morphine and pethidine. As the drug, methadoni hydrochloridum, this opioide is synthetic analgetics and an derivative of heptanone.[1] The chain

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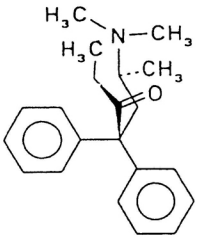

Methadone

Figure 1. Methadone (similarity to morphine).

of etanamine, CH_2*CH_2*$N(CH_3)$- is considered as the holder of the analgesic effect.[2] Methadone is slightly more potent as an analgetic and has slightly weaker sedative action, but considerably higher respiratory depressant and antitussive action than morphine.[3] In addition, methadone exerts hypnotic and euforic action.[2] Unlike morphine, methadone is almost as active by mouth as by parenteral administration.

Heroin (»Bayer«) is a diacetylic ester of morphine (diacetyl morphine). It deacetylises in the body. Diacetyl morphine mostly resembles in action to morphine. The danger of dependence (heroinismus) is extraordinary high[2], but in many countries, heroin became the generally used opioid. Since diacetyl morphine has no therapeutics advances towards morphine, its medical therapeutics use has been abandoned and sale prohibited. In medical practice nowadays methadone finds especial value in the treatment of morphine (and/or heroin) dependence.[4] Prolonged use gives rise to tolerance and dependence of morphine type. These two drugs show cross tolerance, so it is possible to substitute methadone for morphine without precipitating the abstinence syndrome. Methadone can be given before abstinence from morphine. It has been established and is often usual to replace the short acting opiates during withdrawal period to reduce the severity of reactions. Fairly dosage (30–120 mg in man) progressively reduced over 3 weeks and then ceased completely. This regime lowers the degree of tolerance and physical dependence to the point where whithdrawal reactions are mild and do not provide any undue stress.[3] The initial drug treatment of a dependent individual usually involves substitution for morphine, or another drug of the same type, but one, which induces a less unpleasant withdrawal syndrome. Methadone is often used for this purpose, and itself can then be cautiously withdrawn. Because of its longer duration of action (half-life of methadone is 40–90 hours), the methadone withdrawal syndrome reaction is relatively slower in onset, much less longer in duration (reaches plateau rather than peak) and much less intense.[3]

Unfortunately, illicit drug use is widely and rapidly spreading in the developing world. In order to cure abused patients (abusers) pharmacoterapeutics treatment – as methadone, is widely used. However, the mechanism how methadone acts, as well as the consequences, even in healthy subject, are still poorly understood.

Many of neurophysiological impairments are the consequence of the developing diabetes in the experimental conditions.[5,6,7a,7b] Some reports point to the metabolic perturbations produced by hyperglycemia in the developing experimental diabetes as an causative factor for the abnormalities appearing in the somatosensory evoked potential (SEP) wave components.[6,7a,7b]

Somatosensory evoked potentials (SEPs), elicited by electrical pulses, are generated in the afferent pathways of the nervous system and somatosensory cortex of the brain. Recorded electrically from the surface electrodes, they represent the summed neural activity as a complex waveform with several well defined peaks and valleys, which reflect the sensory information processing. Any damage or dysfunction of the nerve cells, connections and white matter to various degrees, leads to the abnormality in the waveforms of the SEPs.[8]

Methadone as a legal heroin substitute,[9] applied in treating heroin addicts, in order to correct their behaviour disturbances, is probably chosen, among others, because of its beneficial effectiveness on the central nervous system (CNS).

The aim of our investigation was to explore if methadone is equally suitable (*i.e.* not additionally harmful) in the treatment of diabetic addicts. Therefore, in the present study we investigated the consequences of 64 days methadone treatment on the somatosensory evoked potentials (SEPs) in diabetic female rats as compared to healthy control of diabetic addicts.

MATERIAL AND METHODS

Female Wistar rats from the breading colony of Rugjer Bošković Institute, Zagreb, Croatia, in the age of 6 months and 290 +/– 30 g of body weight, were used in experiments. Four experimental groups were followed:

– untreated healthy rats,
– healthy rats treated with methadone,
– diabetic rats,
– diabetic rats treated with methadone.

Methadone as methadoni hydrochloridum (Heptanon, »Pliva«, Zagreb, Croatia) was generously gifted from doc. dr. S. Sakoman, Center for the

Study and Prevention of Alcoholism and other Addictions, Clinical Hospital »Sisters of Mercy«, Zagreb, Croatia. It was applied by esophageal tube to the rats, in the dose of 0.87 mg/kg daily, during the period of 64 days.

Experimental diabetes was induced by the single *i.v.* injection of alloxan monohydrate »Sigma«, U.S.A.) in the dose of 65 mg/kg. Glycosuria (urine strips, Ames, U.S.A.), body weight and blood glucose were regularly monitored to ascertain diabetic condition. The level of the blood glucose was determined by God Period, »Pliva«, Zagreb, Croatia.

Animals were weighed and anaesthetized by *i.v.* injection (tail vein) of alpha-chloralose in a dose of 72–80 mg/kg and thereafter monitored respiration frequency and constant body temperature (37 +/– 1 °C). SEPs were evoked by electrical stimulation of the contralateral forepaw subcutaneously and recorded from the scalp, above the underlying somatosensory cortex with the reference needle electrode placed medially to the neck.

Peripheral stimulation consisted of singular rectangular pulses of 0.5 ms duration and 0.3 Hz frequency (SD5 »Grass« stimulator, U.S.A.); stimulus strength was adjusted supramaximally. Specially constructed two channels system for signal recording and analysis contains PC-AT and multifunction DT2801 A/D converter board. The instrumentation used and the more detailed description of the computorized program, with adapted structures of hardware and software to satisfy the specific demands, necessary for recording of SEPs in small animals, as well as storage and analysis of averaged SEPs (64 in each curve) was reported elsewhere.[7a] SEPs were measured repeatedly in the 15 min intervals through at least 60 min time period of each animal.

Statistical evaluation of the results were performed using one way analysis of variance ANOVA) followed by Newman-Keuls test, as a method of multiple comparation, or by Student's *t*-test, where appropriate. Significance was accepted when P values were less than 0.05.

RESULTS

SEPs recorded from the scalp of the transiently anaesthetized untreated healthy rats, using an non invasive technique,[7a] represent the typical and reproducible N1, P2, N2 and P3 wave complex shown in the upper right corner of the the Figure 2. They are defined by its latencies and amplitudes (Figure 2 and Table I). Characteristic wave deflections are alternatively named in the further text as N20 for N1, P25 for P2, N28 for N2 and P30 for P3 according to the latency of their peaks (Figure 3). The basic shape of the N1, P2, N2 and P3 wave complex remained, and could be followed in the three groups of differently treated animals as well (Figure 2).

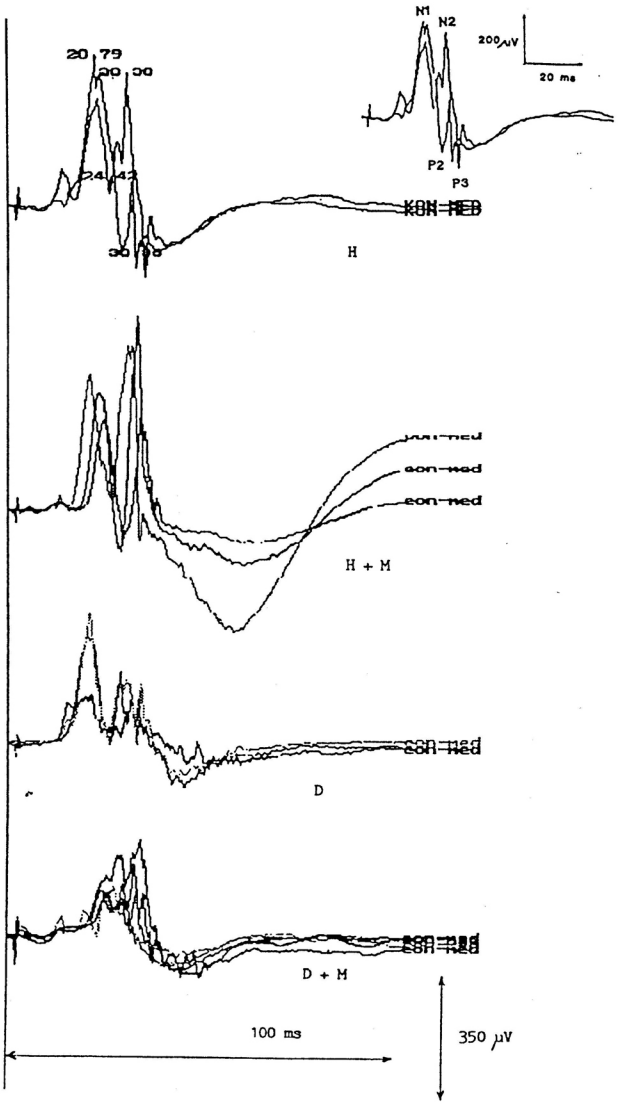

Figure 2. Somatosensory evoked potentials (SEPs) in two months methadone (M) treated healthy (H), or diabetic (D) female rats. Upper right corner : wave form of the SEPs recorded from a healthy control rat showing four prominent peaks defined by their latency: first negative (N1, *i.e.* N20), second positive (P2 – P 25), third negative (N2 – N28) and fourth positive peak (P3 – P30). N1 (H) identifies decrease of N1 and P2 peaks in diabetic rats compared to healthy ones regardless the treatment. N2 denotes the marked amplitude enhancement following methadone treatment in both, healthy and diabetic rats. P3 identifies delayed and broadened peak in healthy rats under methadone influence as well as in diabetic untreated rats.

TABLE I

Absolute values of latencies (A) and amplitudes (B) of SEPs in two months methadone(M) treated healthy (H), or diabetic (D) female rats (expressed as a mean +/– standard error of the mean).

(A)

Latency (ms)	Wave components of SEPs			
	N1	P2	N2	P3
H	20.69+/–	25.94+/–	28.43+/–	30.57+/–
	0.234 (7)	0.409 (7)	0.477 (7)	0.649 (7)
H + M	20.66+/–	24.55+/–	28.58+/–	47.56+/–**
	0.757 (5)	1.092 (5)	1.160 (5)	5.018 (5)
D	20.88+/–	24.84+/–	28.76+/–	42.05+/–**
	0.667 (7)	0.573 (7)	0.491 (7)	1.138 (7)
D + M	22.68+/–	24.31+/–	28.11+/–	42.03+/–
	0.983 (7)	0.707 (8)	0.961 (6)	1.354 (6)

(B)

Amplitude (μV)	Wave components of SEPs			
	N1	P2	N2	P3
H	–383.52+/–	+43.42+/–	–214.99+/–	+66.44+/–
	31.678 (7)	19.519 (7)	0.649 (7)	27.709 (7)
H + M	–347.14+/–	+173.30+/–**	–396.45+/–*	+154.46+/–
	83.109 (5)	62.328 (5)	76.561 (5)	40.900 (5)
D	–223.27+/–**	–52.14+/–*	–180.11+/–	+58.76+/–
	31.737 (7)	11.770 (7)	22.410 (7)	11.734 (7)
D + M	–158.33+/–	–117.33+/–	–192.09+/–	+43.48+/–
	20.998 (7)	17.567 (8)	23.614 (6)	7.175 (6)

Methadone produced the marked influence on the SEPs. Highly significant changes were observed in healthy animals after 64 days of every day methadone application by the means of oesophageal tubing. They were characterized by the P3 latency prolongation (156% *vs.* healthy control; 100%), as well as the amplitude enhancement (370% of P2 and 254% of N2; Table II and Table I) as compared to untreated female rats.

Experimental diabetes, provoked by the single alloxan injection, induced significant differences in both, latency and the amplitude of the SEPs. As compared to healthy rats P3 latency delay (138%),accompanied by overall amplitude decay of N1 (58%) and P2 components (55%), was obtained (Figure 2, Figure 3 and Table II). Later components (N2, P3) remained unaffected (Table II).

Two months methadone treatment of diabetic rats induced augmentation of P2 (P25) amplitude (225% *vs.* diabetic control; Table II).

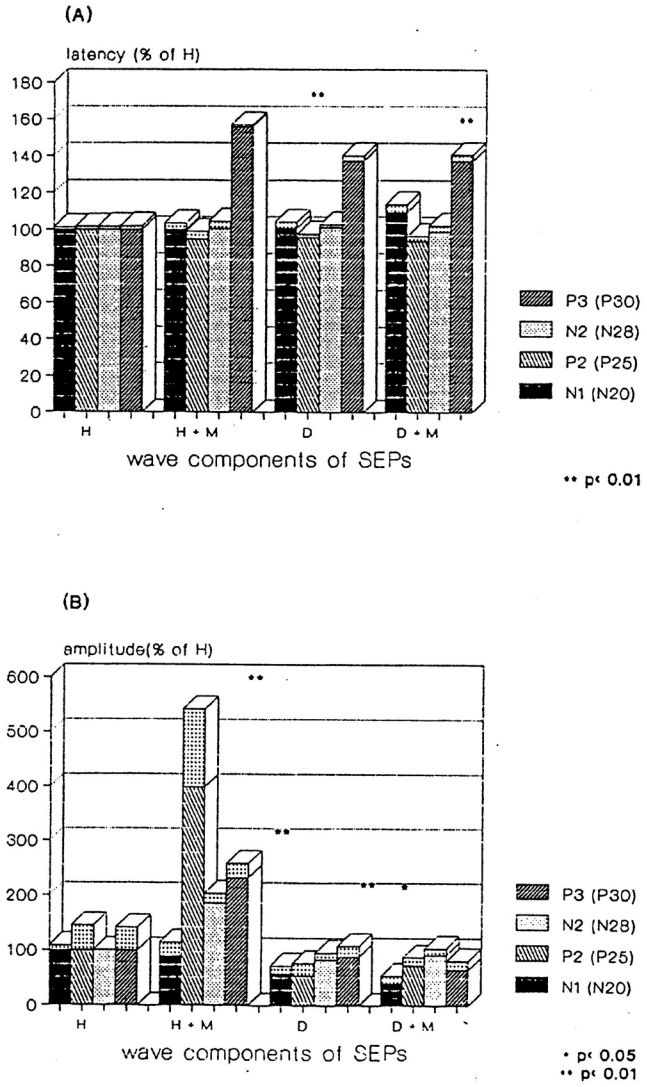

Figure 3. Percent of latencies (A) and amplitudes (B) of SEPs in two months methadone (M) treated healthy (H), or diabetic (D) female rats (expressed as a mean +/– standard error of the mean).

There was no difference in the effect of methadone on the latency for both groups of rats, *i.e* healthy *vs.* diabetic one, but marked amplitude decrease of the first three components of SEPs investigated in the experiments presented in this study (N1, P2 and N2) in diabetic rats, was observed (Table II).

TABLE II

Relations of SEPs components parameters (latencies and amplitudes) in two months methadone (M) treated healthy (H) or diabetic (D) female rats

Latency (%)	Components of SEPs			
	N1	P2	N2	P3
H : (H+M)	ns	ns	ns	** ↑ 155.87
D : (D+M)	ns	ns	ns	ns
H : D	ns	ns	ns	** ↑ 137.55
(H+M) : (D+M)	ns	ns	ns	ns
Amplitude (%)				
H : (H+M)	ns	** ↑ 370.46	* ↑ 253.78	ns
D : (D+M)	ns	* ↑ 225.04	ns	ns
H : D	** ↓ 58.2	* ↓ 54.56	ns	ns
(H+M) : (D+M)	* ↓ 45.61	** ↓ 40.37	* ↓ 48.45	ns

DISCUSSION

Our present findings in alloxan treated female rats 6 months of age, point to central effect, since the components of SEPs exceeding latency of 20 ms were involved, as it was the case in our previous study.[7a] Therefore, it might be supposed that the amplitude decrease might be attributed to some kind of disturbances in triggering the neurons in higher structures.[7a] Methadone, given every day to the rats by oesophageal tube, had no harmful influence in diabetic animals. On the contrary, it showed some kind of benefitial effect, since not only total recovery of the SEPs amplitude (P25) occurred, but the marked enhancement of P2 wave was seen. Recently, we observed that in case of AST and ALT enzyme activity methadone exerts similar beneficial effect, or at least, does not deteriorate already existing diabetic condition.[10]

On the basis of our investigation, we concluded that chronical treatment of methadone caused enhancement of the SEPs with the latency prolongation of later components in both, healthy and diabetic rats (Figure 2), although in the latter, with slightly lower potency. This means that no essential differences exist in the effects of methadone on healthy in comparison to diabetic subjects. Therefore, any harmful effect of methadone is not to be expected, what is important in connection to with methadone substitution therapy of abusers.

Moreover, the amplitude enhancement (225%) in diabetic, methadone treated rats, actually point to a certain recovery process as suggested by N2 change.

Alloxan causes diabetes in experimental animals through its ability to destroy insulin secreting beta-cells of pancreas.[11] It has been reported that fibre diminution and the decreased axon/myelin ratio may explain the slowing nerve conduction in experimental diabetes,[12a] although no segmental demyelinization or remyelinization was found, but the nodes of Ranvier were slightly widened and paranodal bulbi were swollen relative to fibre calibre.[12b] Since nerve conduction studies involve stimulation of either motor, or sensory nerves with subsequent recording of either a compound action potential, evaluation of several parameters including latency, conduction velocity and amplitude is helpful in determining the type of fibre involvement. The amplitude of the evoked response is a function of number and size of nerve fibres and may be decreased in axonopathies. Reduction of amplitude of SEPs has been shown to be very important in detecting early nerve involvement.[8] However, although there are reports which point to metabolic disturbances developed in experimental diabetes as a causative factor for abnormal auditory,[13] visual[6] and somatosensory[7a,7b] evoked potentials, it is still unclear whether these abnormalities accompanied by decreasing the amplitude of evoked potentials in rats[7a] and prolongation of their latency,[6] are the result of peripheral nerve involvement, or they represent central involvement, or both. Synaptic abnormalities must also be taken in account.[6]

At last, but not the least, with our SEPs recording technique, next to cortical waves, additionaly, we collect the numerous data which could be available for use any time later. It is important that with our noninvasive method we can follow the SEPs in the course of animal disease at any time, as well as record them numerically.[14]

After acute treatment of rats with morphine Abdulla and Aneja[15] recorded SEPs from the skull overlying the contralateral somatosensory area of the cortex. They observed that components I and II of subcortical SEPs were not significantly affected. But, the amplitude of component III was inhibited (up to 50%). This is contrarily to our observations with methadone. However, in the first place, our treatment lasted two months, and second, it might be that the methadone and morphine action on SEPs are different. Namely, it was believed that analgetics have little or no effect on specific sensory cortical SEPs.[16] Large doses of morphine (5–10 mg/kg) and methadone enhanced auditory, visual and somatosensory EPs in the primary projection areas and produced various changes in other cortical regions.[16]

Methadone treatment is widely used as a substitute in the treatment of opioid dependent patients[17–19] since it prevents opiate withdrawal symptoms for 24 to 36 hours. Methadone treatment of diabetic rats, according to the presented data, provide the normalization of SSEPs opposing to their impaired quality developed in diabetes.

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SAŽETAK

Evocirani potencijali u dijabetičnom sindromu štakorica prije i nakon dvomjesečne terapije metadonom

Milica Bjegović, Velimir Išgum i Milivoj Slijepčević

Istražen je učinak dvomjesečne terapije metadonom (0.87 mg/kg sondom per os; svakodnevno) na somatosenzorne evocirane potencijale (SEP) u kontrolnih (zdravih) i dijabetičnih 6-mjesečnih štakorica. Dijabetično stanje inducirano je jednokratnom injekcijom aloksan monohidrata (65 mg/kg *i.v.*). SEP dobiveni nakon električne stimulacije kontralateralne šapice i registrirani iz skalpa, putem neinvazivne metode, pokazali su visoku reproducibilnost. Produžena latencija (156%) i povećana amplituda

(371%) karakteriziraju somatosenzorne potencijale tzv. srednjih latencija, nastalih svakodnevnom primjenom metadona tijekom 64 dana u zdravih štakorica. Eksperimentalni dijabetes signifikantno mijenja oba ispitivana parametra; reducira amplitudu ranijih (N1, P2) i produžuje latenciju kasnijih komponenata SEP-a (P3). Dvomjesečno izlaganje dijabetičnih štakorica metadonu ne samo da oporavlja, već i dodatno povećava amplitudu P2 (P25) valne komponente SEP-a (225%). Rezultati ukazuju da nema bitnih razlika u djelovanju metadona na SEP izmedju kontrolnih zdravih i dijabetičnih Wistar štakorica.