Pharmacology Biochemistry & Behavior, Vol. 6, pp. 87–92.

Sudden Toxicity of Methadone in Monkeys: Behavioral and Electrophysiological Evidence

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(Received 19 August 1976)

SNYDER, E. W., R. E. DUSTMAN, R. C. STRAIGHT, A. W. WAYNE AND E. C. BECK. *Sudden toxicity of methadone in monkeys: behavioral and electrophysiological evidence.* PHARMAC. BIOCHEM. BEHAV. 6(1) 87–92, 1977. — A sudden and potentially lethal toxic reaction to a previously well-tolerated maintenance dose of methadone occurred in 4 of 6 monkeys. The reaction was characterized by gross behavioral and respiratory depression and a marked attenuation of both early and late components of the visual evoked response with an increase in most latencies. The nature of the evoked response alteration suggests a widespread central nervous system depressant effect of the drug during toxicity. Concomitant with the toxic reactions were dramatic increases in plasma methadone concentrations. Therefore the observed changes in sensitivity to methadone would appear to be the consequence of a sudden shift in pharmacokinetics resulting in toxic plasma concentrations.

Methadone Sudden toxicity Visual evoked response Stump-tailed macaques

WHILE the mechanisms and characteristics of tolerance to narcotic analgesics have been carefully studied [5] the phenomenon of toxicity of a previously tolerated dose has received only passing attention. Early work with monkeys [33] described an increased susceptibility to methadone with repeated administrations of the maximum tolerated dose. This increased susceptibility, often terminating fatally [25], reportedly persists as a semipermanent residue even after complete drug detoxication. However, other than invoking neuropathological origins, no explanation for the phenomenon has been offered [14]. A recent paper [6] suggested that such a reaction to a high maintenance dose may occur when the monkey is weakened by illness, trauma or advanced age. In short the phenomenon has been infrequently observed and inadequately explained. However, all reports of altered susceptibility during maintenance on a narcotic analgesic have been based upon experiments involving doses which, on first administration, caused severe depression in at least some animals [6]. There have been apparently no reports of sudden toxicity in healthy animals maintained on moderate, fixed doses of a narcotic. The present study provides behavioral and electrophysiological descriptions of such a reaction to methadone along with critical plasma levels of the drug in monkeys.

METHOD

Animals and Surgery

Twelve stump-tailed macaques (*Macaca arctoides*) weighing between 5.0 and 6.4 kg were immobilized with phencyclidine HCl (Sernylan), anesthetized with 10–20 mg of sodium pentobarbital (Nembutal) and stereotaxically implanted with stainless steel screw electrodes contacting dura and with depth electrodes positioned according to atlas [28] in critical subcortical structures. Only VERs recorded from striate cortex with reference to frontal sinus will be described in this paper. Electrodes were connected to a small pedestal which was anchored to the skull [26]. Animals were allowed at least 3 weeks to recover from surgery before testing began.

Procedure and Equipment

All animals were confined to plastic restraining chairs for many months prior to and throughout the maintenance period except for a 12 hr exercise period per week when the monkeys occupied separate cages. During recording the restrained animal was positioned with its eyes near the center of a reflecting hemisphere. Thus slight head movement did not change the effective intensity of the light which was 144 lux. White noise was presented throughout testing to mask extraneous sound. A Grass PS22 photic stimulator lamp enclosed in a sound attenuated fiberglass box was positioned behind and above the monkey's head and delivered 10 μsec flashes of light into the center of the hemisphere which was 51 cm distant. VERs were summed from blocks of 50 flashes which were presented at about 2 sec intervals during artifact free periods of EEG. The EEG was amplified by a Grass Model 78B EEG polygraph (bandwidth 0.3–300 Hz, time constant 0.25 sec) and, together with electrical pulses coincident with light flashes, was stored on magnetic tape by a 7 channel Hewlett-Packard tape recorder. One second epochs of EEG following each electrical pulse were digitized at a rate of 500/sec by an AF01-B analog to digital converter interfaced to a DEC PDP-9 computer which summed and averaged VERs. Amplitudes of the regularly occurring VER waves were determined by measuring the vertical distance from the trough

or peak of each wave to the trough or peak of the preceding wave. Latencies to each peak were measured from stimulus onset.

Each recording session began with the restrained animal being placed in a sound-attenuated, electrically shielded room and connected to the EEG located in an adjoining room. The animal's head was held gently in a fixed position by an experimenter sitting behind and to one side of the monkey. This procedure resulted in records which were largely artifact free and permitted close observation of the animal's behavior. The monkeys were closely monitored to insure that their eyes were fully open throughout recording.

Following baseline recording, 6 monkeys began sustained ingestion of methadone with 6 placebo-control animals receiving the cherry flavored syrup used as a vehicle for methadone. All animals were weighed at least weekly (triweekly during the initiation of drug treatment) for dose adjustment purposes. The drug, methadone HCl dissolved in water, was administered orally in cherry syrup at 8:00 a.m., 4:00 p.m., and 9:30 p.m. daily in an effort to keep the animals continually exposed to safe levels of the drug. With rare exception the drug was totally consumed within 1 min of administration.

During the initiation of methadone treatment the total daily dose of 3 mg/kg was increased rapidly to 15 mg/kg/day over a 2 week period and VERs were recorded at least 4 days per week between 12:00 Noon and 1:30 p.m. Maintenance on 15 mg/kg/day began after the second week and continued at this dose until recurrent toxic reactions necessitated reduction in daily dose. Blood samples were drawn immediately after a recording session and plasma was extracted for radioimmunoassay of methadone content (Table 3). Unfortunately with repeated extractions blood samples became more difficult to obtain in the later weeks of maintenance.

Analytical Methods

Existing quantitative assays [30] for methadone in serum or plasma involving elaborate extraction procedures with organic solvents, require 4–10 ml of blood. Since we found it especially difficult to repeatedly obtain samples of this size, we developed a radioimmunoassay with a lower-limit sensitivity of 20 ng/ml, suitable for much smaller (0.1 ml) samples. In brief, we used a commercially available rabbit antiserum (Technam, Inc.) specific for total methadone and cross reacting metabolites in plasma or serum. Methadone standards were prepared from methadone hydrochloride as follows: Approximately 1 g was recrystallized from methanolether and 100 mg dissolved in 1 liter of water. Then 0, 2, 5, 10, 20 and 30 μl were added to 10 ml of methadone-free plasma. The standards represent 0, 2, 5, 10, 20 and 30 ng of methadone per 0.1 ml of plasma. [^{3}H]-Methadone (New England Nuclear Corp.), obtained at 100 mCi/n mole, was diluted with working buffer to 10,000 dpm per 0.1 ml. Stock buffer was sodium barbital-acetate, pH 8.4 (sodium barbital – 7.354 g and anhydrous sodium acetate – 4.857 g in 250 ml water). Working buffer was prepared by mixing 50 ml stock buffer with 900 ml water plus 8.5 g sodium chloride and adjusting the pH to 7.4 with 0.1 N HCl.

Samples were precipitated to separate bound from free [^{3}H]-methadone in the radioimmunoassay mixture with polyethylene glycol 6000 (PEG) solution. The solution was prepared as 25 g dissolved in 100 ml of working buffer. All samples were assayed in duplicate along with appropriate standards and controls with each batch of samples. Samples and methadone antibody were mixed and pre-incubated at room temperature for 15 min. [^{3}H]-Methadone (10,000 dpm) was added to each tube and incubated for 15 min at room temperature followed by incubation for 2 hr at 4°C with mechanical shaking. PEG was added to precipitate bound methadone and bound vs free [^{3}H] methadone was separated by centrifugation. Free [^{3}H]-methadone was determined in a Model 3385 liquid scintillation counter (Packard Instrument Co.). The scintillation counting mixture was permafluor (55 ml, Packard Instrument Co.), naphtalene (150 g) and 2-ethoxy-ethanol (300 ml) in 1 liter of solution with toluene. Sample methadone was determined from a standard curve (percent [^{3}H]-methadone bound vs methadone concentration) prepared for each batch of samples (Fig. 1).

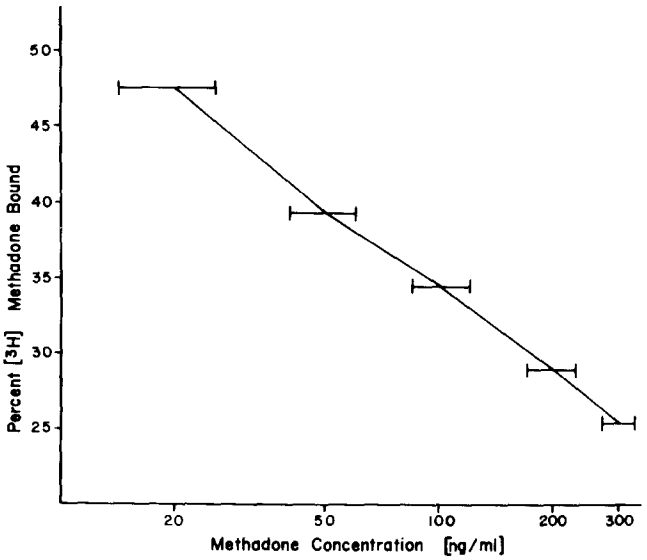

FIG. 1. Mean (n = 19) standard curve and 95% confidence intervals for radioimmunoassay of plasma methadone.

RESULTS

During initiation of methadone treatment and early maintenance neither the monkeys' behavior nor their VER changed appreciably from baseline. However, after less than 3 weeks of methadone ingestion at approximately 2 hr after the a.m. dose (5 mg/kg) Monkey 107 was quite depressed with shallow breathing, gross ataxia, fixed gaze and slightly dilated pupils. The animal's VERs recorded prior to, during and following this first toxic reaction are depicted in Fig. 2.

All VER components showed a decrease in amplitudes (Table 1) and an increase in latencies (Table 2) during the toxic reaction. The majority of these changes were greater than 2 standard deviations (SD) from pre and post-toxic means. The plasma methadone concentration during the animal's first toxic reaction was well above the range of pre and post-toxic values (Table 3).

With no changes in the dosing regimen, Monkey 107 did not have another reaction until the 13th week of maintenance when, for the first time, a reaction required nalorphine HCl (0.5 mg administered subcutaneously) to reverse respiratory depression. The animal recovered quick-

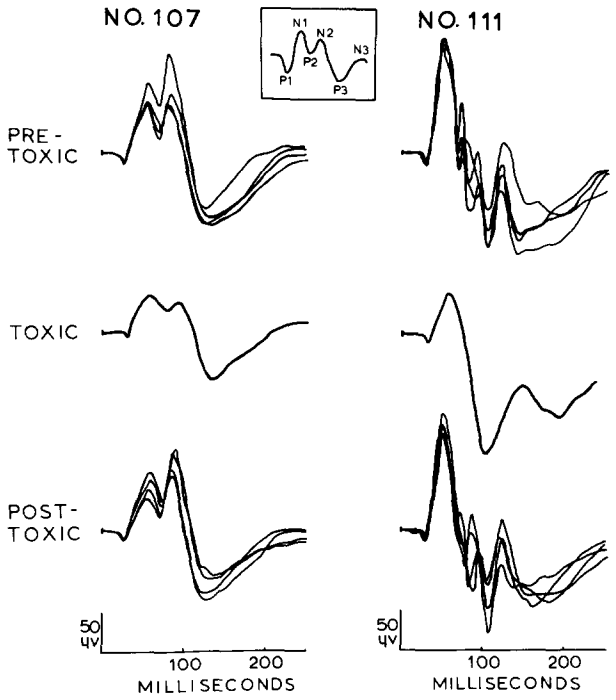

FIG. 2. VERs recorded from straite cortex during a two-week period of methadone maintenance prior to a toxic reaction (pre-toxic), during a toxic reaction, and during a two-week period of continued methadone maintenance following a toxic reaction (post-toxic).

ly and fully. However, it was not possible to obtain either VER records or a blood sample following this reaction (Table 3).

Another animal (No. 111) first showed the reaction after more than a month of methadone ingestion. During toxicity the amplitudes (Fig. 1, Table 1) of most components were below (>2 SD) pre and post-toxic means. In fact, P2-N2 disappeared entirely. All latencies, except that of P3, increased (>2 SD) beyond pre and post-toxic means with the latency of peak N3 showing the largest increase (Table 2). During this reaction the animal showed a methadone plasma level well above the range of pre and post-toxic values (Table 3). One hour after the first VER recording a second record and a blood sample were obtained showing a partial return of P2-N2 and a plasma methadone level of 165 ng/ml. During all subsequent toxic episodes Monkey 111's plasma methadone concentration was well above those values obtained in the absence of toxicity (Table 3). VERs obtained during the second and

TABLE 1

AMPLITUDES OF VER COMPONENTS FOR TWO MONKEYS PRIOR TO, DURING, AND FOLLOWING TOXIC REACTIONS

Monkey	Condition	Amplitudes (μV) P1-N1	N1-P2	P2-N2	N2-P3	P3-N3
107	Pre-toxic	80.4	32.5	49.0	165.0	*
		(13.0)	(6.8)	(13.5)	(20.0)	
	Toxic	48.0	14.0	6.0	91.0	*
	Post-toxic	75.8	31.9	54.5	158.5	*
		(11.9)	(5.8)	(11.6)	(16.1)	
111	Pre-toxic	146.0	140.3	30.9	117.5	78.0
		(4.5)	(13.0)	(20.8)	(39.0)	(6.7)
	Toxic #1	60.0				80.0
	Toxic #2	56.0				77.0
	Toxic #3	66.0				12.0
	Post-toxic	139.5	142.5	26.5	95.0	78.3
		(10.4)	(18.1)	(25.0)	(35.2)	(9.8)

Pre- and post-toxic means and standard deviations ( ) were derived from four VERs collected over a two-week period. The absence of P2-N2 in Monkey 111's toxic record made it impossible to score N1-P2, P2-N2 and N2-P3.

*Monkey 107 lacked a P3-N3 component (see Fig.2).

TABLE 2

LATENCIES OF VER COMPONENTS FOR TWO MONKEYS PRIOR TO, DURING AND FOLLOWING TOXIC REACTION

Monkey	Condition	Latencies (msec) P1	N1	P2	N2	P3	N3
107	Pre-toxic	27.0	57.8	70.0	83.3	130.0	*
		(.8)	(1.7)	(2.8)	(3.2)	(5.7)	
	Toxic	30.0	60.0	84.0	94.0	133.0	*
	Post-toxic	26.9	57.3	71.0	83.9	129.5	
		(.8)	(1.6)	(2.2)	(3.1)	(5.8)	*
111	Pre-toxic	27.0	48.8	65.8	71.5	103.3	120.3
		(.8)	(1.3)	(1.7)	(1.3)	(1.0)	(2.2)
	Toxic #1	31.0	57.0			104.0	148.0
	Toxic #2	31.0	56.0			108.0	152.0
	Toxic #3	32.0	56.0			112.0	153.0
	Post-toxic	28.0	49.8	71.0	78.3	104.0	121.6
		(0.0)	(1.0)	(5.2)	(7.8)	(.8)	(1.5)

Pre- and post-toxic means and standard deviations ( ) were derived from four VERs collected over a two-week period. P2-N2 was absent in Monkey 111's toxic record.

*Monkey 107 lacked an N3 component (see Fig. 2).

TABLE 3

PLASMA LEVELS OF METHADONE PRIOR TO, DURING AND FOLLOWING EACH TOXIC EPISODE

			Plasma Level (ng/ml)								
			Pre-toxic				Post-toxic				
Monkey	Sex	Episode No.	$\bar{X}$	Range	N	Toxic Value	$\bar{X}$	Range	N	Week	Dose
103	F	1	23	20-85	27	250	25	20-100	10	24	12
104	M	1	29	20-140	30	—	20	20-35	6	28	10
107	F	1	32	20-60	5	220	70	20-100	3	3	15
		2	70	20-100	3	—	22	20-50	7	13	15
111	F	1	54	20-100	10	230	31	20-100	13	5	15
		2	31	20-100	13	130	—	—	—	12	15
		3	—	—	—	210	70	40-105	14	13	15
		4	70	40-105	14	420	30	—	1	19	12
		5	30	—	1	175	—	—	—	20	12
		6	—	—	—	250	*			21	12

Monkeys 107 and 111: Post-toxic mean ($\bar{X}$), range and number of samples (N), were used as the pre-toxic information for the succeeding episode. Right-hand columns indicate week of maintenance when each episode occurred and the maintenance dose (mg/kg/day) at that time. Given the lower limit sensitivity of the assay all values of ≤20 ng/ml are referred to as 20 ng/ml. Data not available—.

*At three hours following Monkey 111's final episode its plasma level had dropped to 150 ng/ml, at six hours to 76 ng/ml and on the following A.M. to ≤20 ng/ml where it remained until the animal's death.

TABLE 4

PLASMA LEVELS IN ANIMALS NOT EVIDENCING TOXICITY

				Plasma Levels (ng/ml)		
Monkey	Sex	Weeks of Maintenance	Dose (mg/kg/day)	$\bar{X}$	Range	N
102	M	2-13	15	37	20-105	8
		14-24	12	24	20-37	4
		25-28	10	20	20-89	5
113	F	2-13	15	29	20-100	9
		14-24	12	28	20-50	3
		25-28	10	35	20-123	5

Mean ($\bar{X}$) and range derived from N samples.

third episode closely paralleled the initial toxicity record (Tables 1,2). However it was not possible to obtain VER records during the later episodes. As the reaction recurred, twice requiring 0.5 mg of nalorphine to reverse respiratory depression, the maintenance dose for all animals was gradually reduced. Despite this precaution Monkey 111's final toxic reaction progressed rapidly into respiratory arrest. Nalorphine restored breathing but the animal suffered severe brain damage and died 3 days later.

After 24 weeks of methadone maintenance Monkey 103 had its only toxic reaction with a blood level of 250 ng/ml (see Table 3). VERs were not recorded at the time of this reaction. The animal recovered fully without nalorphine and showed no further toxic reactions. Monkey 104 had a severe reaction after 28 weeks on methadone when the maintenance dose was 10 mg/kg/day (Table 3). The animal recovered fully with nalorphine (1 mg) and artificial respiration. However, it was not possible to obtain a blood sample or VER record. Following Monkey 104's reaction in the 28th week the dose for all animals was adjusted to reach a final maintenance dose of 7 mg/kg/day with no subsequent toxic reactions.

Two monkeys (Nos. 102 and 113) never evidenced toxicity. Over the weeks of methadone maintenance these animals showed no consistent change in their plasma methadone levels (Table 4).

Thus, four monkeys on sustained ingestion of methadone, at doses typically causing no depression, occasionally evidenced moderate to severe reactions while no control animal ever evidenced a similar reaction either behaviorally or electrophysiologically. Despite gradual decreases in maintenance dose the reaction recurred with one resultant fatality. The reactions were sudden, unpredictable, and occurred with no apparent precipitating factors such as illness, trauma or decrease in body weight. In all instances toxicity occurred within 3 hr following the morning dose of methadone and whenever nalorphine was administered respiratory depression was reversed usually within 3–4 min.

DISCUSSION

While there are apparently no reports of sudden toxicity of methadone in human subjects there is evidence that, with constant daily dosage, individuals show wide fluctuations from day to day and from week to week in plasma methadone [13]. However, these authors report no relationship between symptom complaints and methadone plasma level and conclude that methadone plasma levels do not accurately reflect (at least on a short-term basis) the effective concentrations of methadone at the receptor sites. This conclusion finds little support in the present study where toxicity occurred coincident with elevated plasma levels of methadone.

Although the mechanism of sudden shifts in plasma methadone concentration remains obscure, recent work

with mice [20] suggests one plausible explanation. If enzyme induction is involved in tolerance to methadone, and there is evidence that it is [29], then elevated plasma levels could result from the disruption of this system due to temporary changes in nutrition, ambient temperature or general cleanliness of the environment [32]. In the present study animal maintenance and recording procedure were standardized and no environmental fluctuations coincident with toxicity were detected. However, this is not to say that such fluctuations did not occur.

Another explanation for toxicity is suggested by the evidence for a reservoir of methadone in tissue [7]. Perhaps fluctuations in plasma levels reflect unpredictable shifts in the equilibrium between reservoir and plasma methadone.

Although the behavioral signs of toxicity were unmistakable, a more quantitative description is provided by the VER. It is useful to divide the VER into primary (0–70) and secondary (post 70 msec) components since there is general agreement that the primary components reflect activity of the classic afferent system ascending through specific thalamic areas to primary receiving cortex while the secondary components are apparently influenced by collaterals from the reticular activating system and nonspecific thalamocortical pathways [1,4]. With this differentiation various drugs have been classified and described in terms of their probable site of action.

Barbiturates and inhalation anesthetics at doses close to and even above those which produce unconsciousness do not attenuate and may even enhance primary components while attenuating secondary components of the evoked response recorded from striate cortex. The primary components typically remain resistant to these drugs until much higher doses are administered [1, 4, 8]. Alcohol apparently has little effect on responses recorded from striate cortex while decreasing the amplitude of secondary components in responses recorded from associational cortex. Again the mesencephalic reticular formation and nonspecific thalamortical pathways have been implicated as principal sites of the drug's effect [17, 22, 24]. The findings with marijuana are inconclusive [18, 19, 23], however the drug does not appear to act on brain-stem centers as do the general anesthetics and alcohol.

Narcotic analgesics, in moderate doses, apparently enhance primary and secondary components of the evoked response in cats and dogs [3,9]. While the mechanism of such enhancement is not known, a number of possibilities including a direct stimulant effect, depression of an inhibitory system and a reduction in spontaneous activity of relay neurons have been suggested [3]. There is, however, some indication that the diffuse thalamocortical projection system is depressed by morphine in dogs, cats and rabbits [16, 21, 27]. Given the considerable species differences in the response to narcotic analgesics and the absence of adequate dose response information it is not surprising that no consistent drug-effect pattern emerges. The results of the present study indicate that methadone, at plasma levels which produce behavioral depression without loss of consciousness, attentuates all VER components and increases their latencies suggesting a generalized depression of the primate visual system from brain stem to cortex. While more peripheral (e.g. retinal) effects may have contributed to the VER alteration [1] the effects of methadone were exactly opposite to those of simple pupillary dilatation [10].

On one occasion Monkey 104 had a plasma level of 140 ng/ml without showing signs of toxicity. However, in all other instances a toxic reaction occurred whenever the plasma level of methadone reached or exceeded 130 ng/ml. Since human methadone addicts frequently show blood levels far in excess of those causing severe problems in our monkeys [15] our data indicate that there is a marked difference between human and monkey sensitivity to methadone. In addition, our results support evidence that the ratio of methadone plasma level to oral dose is much lower in monkeys [6] than in man, both upon acute and chronic administration [31]. Apparently the drug is absorbed more readily from the gastrointestinal tract of humans.

Despite possible species differences in drug sensitivity and rate of absorption, any indication of sudden, potentially lethal toxicity has obvious implications for methadone maintenance programs. There are, however, no reports that such a reaction is responsible for methadone related deaths in humans. Such deaths are usually attributed to factors such as acute overdose, the synergistic action of multiple drugs, impaired liver function or loss of tolerance following abstinence and subsequent overdose [2, 11, 12]. If sudden toxicity as described above was a contributing factor in any of these deaths it would almost certainly remain obscure and confounded by a complex and often incomplete medical history.

ACKNOWLEDGEMENT

Supported by the Medical Research Service of the Veterans Administration, project number 0864-05, and by the U. S. Public Health Service Research Grant DA00388 from the National Institute of Mental Health. We thank Mr. Don Shearer, Mr. Paul Sine and the employees of the VA Animal Research Facility for their valuable technical assistance.

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