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The relation between the effect of a subhypnotic dose of thiopental on claw pain threshold in rats and adrenalin, noradrenalin and dopamine levels

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Abstract: Thiopental sodium (TPS) needs to be applied together with adrenalin in order to establish its analgesic effect in general anesthesia. We aimed to investigate the effect of TPS on the claw pain threshold in rats and evaluated its relationship with endogenous adrenalin (ADR), noradrenalin (NDR), and dopamine (DOP) levels. Intact and adrenalectomized rats were used in the experiment. Intact animals were divided into the following groups: 15 mg/kg TPS (TS), 0.3 mg/kg ADR+15 mg/kg TPS (ATS) and 0.3 mg/kg ADR alone (ADR). Adrenalectomized animals were divided into the following groups: 15 mg/kg TPS (A-TS), 0.3 mg/kg ADR+15 mg/kg TPS (A-ATS) and 0.3 mg/kg ADR alone (A-ADR). Claw pain threshold and blood ADR, NDR, and DOP levels were measured. The TS group's claw pain threshold was found low. However, the claw pain thresholds of the ATS and ADR groups increased significantly. In the A-TS group, the pain threshold decreased compared with normal, and in the A-ATS and A-ADR groups, the pain threshold increased. TPS reduced the blood ADR levels in intact rats; however, no significant changes were observed in the NDR and DOP levels. #TPS provides hyperalgesia by reducing the production of ADR in rats. The present study shows that to achieve analgesic activity, TPS needs to be applied together with ADR.
Key words: catecholamines pain, rat, thiopental sodium

Introduction

Thiopental is a barbiturate-type drug [9]. Barbiturates are used as parenteral anesthetic agents [15]. Thiopentals are the most widely used barbiturate-type general anesthetic agents in animal experiments [6]. Thiopental is generally used in hypnotic form in the induction of general anesthesia [15]. Since thiopental does not cause arrhythmia, it is often preferred in the design of experiments concerning the cardiovascular system in particular [6]. Hypnosis, muscle relaxation, analgesia, and elimination of responses to painful stimuli are some of the most important elements in quality anesthesia [13]. In addition, drugs used in general anesthesia should have no side-effects or the side effects should be reduced to a minimum. However, depression and decreased cardiac output and systemic blood pressure are seen during thiopental use [12]. These effects, which can be easily compensated for in the normal cardiovascular system, can lead to serious complications in the elderly and individuals with an atherosclerotic heart. Thiopental has been shown experimentally to suppress catecholamine

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production. The side effects of thiopental, such as bronchospasm and a decrease in cardiac output and blood pressure, suggest that they may originate from the suppression of endogenous catecholamine production. Thiopental has also been reported to induce hyperalgesia [2]. This has restricted its use apart from in minor, non-painful procedures [26]. Tatsuo *et al.* reported that thiopental induced hyperalgesia [23]. Ketamine, an intravenous anesthetic material, possesses analgesic activity. Ketamine has been reported to raise the levels of adrenalin (ADR), noradrenalin (NDR), and dopamine (DOP) [10, 24]. It has therefore been suggested that the side effects of ketamine, such as a rise in blood pressure and tachycardia, originate from its sympathomimetic activity [25]. This information from the literature suggests that the analgesic effect of ketamine may be related to catecholamine stimulation and that its hyperalgesic effect may be related to inhibition of endogenous ketamine production. Studies have reported that ADR, a catecholamine, exhibits analgesic activity, and that it exhibits this activity via the β_2 adrenergic receptor [17]. This shows that thiopental's lack of analgesic activity and the fact that it causes algesia may be associated with its reduction of catecholamine production. The purpose of this study was therefore to investigate the effect of a subhypnotic dose of thiopental on the claw pain threshold in rats and to assess the correlation of this with endogenous catecholamines (ADR, NDR, and DOP).

Material and Methods

Animals

All procedures were approved by the Ataturk University Institutional Animal Care and Use Committee, and all studies were performed in accordance with the ethical guidelines set out by the local ethical committee which were fully compatible with theNIH Guide for the Care and Use of Laboratory Animals. The protocols and the procedures were approved by the local Animal Experimentation Ethics Committee (date: 25 october 2013, meeting no. 5, decision no. 125). The male albino Wistar rats to be used in the experiment were obtained from the Atatürk University Medical Experimental Application and Research Center. Sixty rats weighing between 220 and 230 g were used. Animals were kept in groups at normal room temperature 22°C, and the relative humidity was maintained at approximately 50 to 60% under appropriate conditions in the laboratory of our pharmacology Department for 3 days before the experiment.

Chemical substances

The thiopental sodium used in the experiment was obtained from I.E. ULAGAY (Turkey), and the adrenalin was obtained from Galen (Turkey).

Animal groups

1) Intact animal groups

TPS salt was used in the experiment. Intact animals were divided into groups given 15 mg/kg TPS (TS) (n=10), 0.3 mg/kg ADR + 15 mg/kg TPS (ATS) (n=10) or 0.3 mg/kg ADR alone (ADR) (n=10).

2) Adrenalectomized animal groups

Adrenalectomized animals were divided into groups given 15 mg/kg TPS (A-TS) (n=10), 0.3 mg/kg ADR + 15 mg/kg TPS (A-ATS) (n=10) or 0.3 mg/kg ADR alone (A-ADR) (n=10).

3) Experimental protocol

All the measurements using Basile Analgesy-Meter (Randall-Selitto method) were perfomed by a single blinded researcher who did not know which treatment protocol group belonged to in the framework of defined systematic criteria.

4) The effect of thiopental sodium on claw pain threshold in intact rats

In this part of the experiment, the claw pain thresholds of rats were measured using a Basile Analgesy-Meter before drugs were given to the TS, ATS, and ADR groups [5]. The rats were held for an hour not to increase the precision which was performed on pressure of paw tissue. Next, TPS at a dose of 15 mg/kg was administered intraperitoneally (i.p.) to the TS group. The ADR group was injected i.p. with 0.3 mg/kg ADR. Five mins after injection of the ATS group with 0.3 mg/kg ADR i.p., TPS was administered ip at a dose of 15 mg/kg. Claw pain thresholds of all animal groups were remeasured 15 min after administration of drugs. Drugs were administered to rats one-min intervals so that time-lost during the measurement period could not modify the action of drugs. This one-min period was determined by a preliminary study. The paw pain thresholds of the animals were measured immidiately after the blood samples were taken from their tail veins of them to determine the adrenaline, noradreanline, and dopamine levels, and the results were compared among the groups.

5) The effect of thiopental sodium on claw pain threshold in adrenalectomized rats

Table 1. The effect of thiopental sodium on claw pain threshold in intact and adrenalectomized rats

Groups	Pain threshold (g)		*P**	Algesia (%)	Analgesia (%)
	Before drugs	After drugs			
TS (n=10)	26.1 ± 1.2	19 ± 1.2	<0.05	27.2	
ATS (n=10)	24.3 ± 1.4	39.8 ± 2.2	<0.01	-	39
ADR (n=10)	24.8 ± 4.9	39.1 ± 2.2	<0.01	-	36.6
A-TS (n=10)	18.3 ± 2.5	16.8 ± 2.6	>0.05	20.1	-
A-ATS (n=10)	19.5 ± 0.9	34.2 ± 1.3	<0.01	-	43
A-ADR (n=10)	16.3 ± 2.4	28 ± 2.5	<0.01	-	41.8

The paired *t*-test test was performed. TS, intact groups receiving 15 mg/kg thiopental sodium, ATS, intact groups receiving 0.3 mg/kg adrenaline + 15 mg/kg thiopental sodium, ADR, intact groups receiving 0.3 mg/kg Adrenaline; A-TS, adrenalectomized groups receiving 15 mg/kg thiopental sodium, A-ATS, adrenalectomized groups receiving 0.3 mg/kg Adrenaline + 15 mg/kg thiopental sodium, A-ADR, adrenalectomized groups receiving 0.3 mg/kg adrenaline; n, number of animals. * $P \leq 0.05$ was significant.

Surgical procedures were performed on rats under sterile conditions with 25 mg/kg intraperitoneal Pentothal anesthesia in a suitable laboratory. So the rats would feel no pain, a single dose of 0.05 mg/kg buprenorphine was injected subcutaneously. The rats were made to stand for the appropriate surgical intervention period after injections. The motionless period of the animals in the supine position was suggested to be the appropriate period for a surgical intervention [7].

All adrenalectomies were done bilaterally through two dorsolateral midflank skin and muscular incisions After reaching the kidneys, the adrenal glands of the animals were dissected with surgical scissors. The incisions were sutured with sterile threads. The rats were fed with 1% sodium chloride and pellet feed instead of water for seven days after adrenalectomy. On the eighth day, the effect of thiopental on rat paw pain threshold was evaluated in adrenalectomized rats [18]. In this part of the experiment, the A-TS group was administered TPS at a dose of 15 mg/kg. The A-ADR group was given 0.3 mg/kg ADR. Five mins after injection of the A-ATS group with 0.3 mg/kg ADR, TPS was injected at a dose of 15 mg/kg. All drugs were given i.p. The A-TS, A-ATS, and A-ADR groups's claw pain thresholds were measured using a Basile Analgesy-Meter before and 5 min after drugs administration. Immidiately after the paw pain thresholds of the animals were measured, blood samples were collected from their tail veins to determine the ADR, NDR, and DOP levels. The results were evaluated by comparing them among the groups.

Biochemical analyses

1) Measurement of adrenaline, noradrenaline, and dopamine levels in rats

Blood samples were collected from rat hearts in 2-ml EDTA vacuum tubes to determine ADR, NDR, and DOP levels. Within 15 min of venesection, the EDTA samples for ADR, NDR, and DOP measurements were placed on ice and centrifuged at 3,500 g for 5 min. After centrifugation, plasma ADR, NDR, and DOP concentrations were measured on an isocratic system using a high-performance liquid chromatography (HPLC) pump (Hewlett- Packard Agilent 1100) (flow rate, 1 ml/min; injection volume, 40 μl; analytical run time, 20 min) and electrochemical detector. We used a reagent kit for HPLC analysis of the catecholamines in the plasma serum (Chromsystems, Munich, Germany).

Statistical analysis

The paired *t*-test test was used to determine the significance of study parameters between groups of samples with the PAWS Statistics 18.0 software (SPSS Inc., Chicago, IL, USA). Significance was set at $P \leq 0.05$. The results are expressed as the mean ± SEM.

Results

Pharmacological findings

As shown in Table 1, a 15 mg/kg dose of TPS lowered the intact rat (TS group) pain threshold in the claw by 7.1 g. This shows algesia corresponding to 27.2%. In the ATS group given ADR, however, TPS raised the claw pain threshold by 15.5 g, establishing analgesia at a

Table 2. The effect of thiopental sodium on blood plasma ADR, NDR and DOP levels in ıntact and adrenalectomized rats

Groups	ADR ng/l			NDR ng/l			DOP ng/l		
	Before drugs	After drugs	*P**	Before drugs	After drugs	*P**	Before drugs	After drugs	*P**
TS (n=10)	691 ± 15	281 ± 13	<0.01	489 ± 33	478 ± 14	>0.05	250 ± 18	265 ± 18	>0.05
ATS (n=10)	677 ± 56	662 ± 70	>0.05	445 ± 57	451 ± 44	>0.05	262 ± 22	252 ± 21	>0.05
ADR (n=10)	658 ± 16	1,071 ± 36	<0.01	438 ± 55	426 ± 60	>0.05	246 ± 29	260 ± 36	>0.05
A-TS (n=10)	-	-	-	635 ± 71	627 ± 76	>0.05	393 ± 55	383 ± 58	>0.05
A-ATS (n=10)	-	438 ± 47	-	639 ± 44	635 ± 29	>0.05	385 ± 25	391 ± 24	>0.05
A-ADR (n=10)	-	471 ± 26	-	606 ± 10	616 ± 47	>0.05	378 ± 33	363 ± 38	>0.05

The paired *t*-test test was performed. ADR, adrenalin; NDR, noradrenalin; DOP, dopamine; n, number of animals. * $P \leq 0.05$ was significant.

level of 39%. In the claw given ADR alone, ADR established analgesia at a level of 36.6%, raising the pain threshold by 14.3 g. Although TPS reduced the pain claw threshold by 1.5 g in adrenalectomized rats, it raised it by 14.7 g in rats given ADR. This corresponds to a 43% analgesic effect. ADR alone in adrenalectomized rats established significant analgesia that corresponded to a level of 41.8% (Table 1).

Biochemical results

As shown in Table 2, TPS reduced plasma ADR levels in intact rats ($P<0.01$), but caused no significant change in NDR and DOP levels. However, the ADR levels in rats given ADR alone were significantly higher compared with those of the rats in the ATS group, which were given ADR together with TPS ($P<0.01$). In addition, while no plasma ADR level could not be measured in adrenalectomized rats, the NDR and DOP levels exhibited a significant increase compared with those in the intact rats. TPS caused no significant change in levels of ADR given externally (Table 2).

Discussion

This study investigated the effect of a subhypnotic dose of TPS on the claw pain threshold in rats and evaluated the relation between the effect and the endogenous catecholamines ADR, NDR, and DOP. The experimental results showed that the effect of TPS on the pain threshold in rats is correlated with ADR levels. This is because there was a significant decrease in endogenous ADR levels in blood plasma in the TS group, which had a significantly low pain threshold.

The decrease in blood ADR levels in rats given TPS may account for how TPS induces algesia. In adrenalectomized rats, the plasma ADR level after A-TS and A-ADR administration may have been lower than the ADL level before drug administration in intact rats. When we compared these adrenalectomized groups with TS group, which showed no analgesic activity, a significantly higher amount of adrenaline was found in the A-ATS and A-ADR groups. So the plasma ADR level after A-TS and A-ADR administration may have been lower than the ADR level before drug administration in intact rats, but a certain concentration level of adrenaline is enough for to occur the analgesic activity. This reveals that the amount of blood ADR should not fall below a certain level for TPS to be able to establish analgesic activity. TPS has also been reported to suppress catecholamine production in the literature [14]. Previous studies have also evaluated an increase rise in pain threshold as an analgesic activity and have reported that ADR induces significant analgesic activity [5]. Although the plasma ADR level after ATS is almost equal to the plasma ADR level before drug administration, ATS showed an analgesic effect. As shown in Table 2, TPS alone reduced adrenaline levels to 281 ng/l, and no analgesic effect was observed. When exogenous adrenaline was administered in the ATS group, the adrenaline level increased to 662 ng/l. To reach the level of adrenaline necessary to create an analgesic effect of thiopental may be provided with exogenous adrenaline. So it can be seen that exogenous ADR in the ATS group maintained sufficient ADR levels to create analgesic effects. Proinflammatory mediators are known to be involved in the development of pain. These include prostaglandins, which increase sensory nerve endings' sensitivity to pain [22]. Prostaglandins are products of the arachidonic acid pathway and are produced by cyclooxygenase (COX) [21]. Prostaglandins increase the sensitivity of C-fiber pain receptors to mechanical and chemical stimuli by reducing the stimulation thresholds of polymodal receptors [3]. The most studied isoforms of the enzyme COX are COX-1 and COX-2. COX-1 is a structural enzyme pres-

ent in an active form in the majority of tissues [16]. COX-2 is involved in the production of proinflammatory prostaglandine, which was induced by proinflammatory agents and prostoglandine increases the pain sensitivity in peripheral tissues. [16, 19]. One study implicated ADR in the recurrence of rheumatic pain on rainy days and in the loss of effect of anti-inflammatory drugs; ADR levels decreased significantly on rainy days compared with sunny days [20]. This information from the literature is compatible with our own study results.

The present study also revealed that the hyperalgesic effect of TPS is not dependent on NDR and DOP. Although NDR and DOP levels in adrenalectomized rats rose significantly compared to those in the intact animals, TPS significantly lowered the pain threshold in adrenalectomized rats. This shows that NDR and DOP are not involved in analgesic activity. We encountered no information in the literature to suggest that NDR leads to hyperalgesia [8]. However, studies have shown antinociceptive activity of NDR in the spinal cord [27].

Our experimental results and the data from the literature are insufficient to show whether NDR and DOP have a definite role in analgesia or hyperalgesia. Nevertheless, this study does show that TPS did not inhibit ADR given externally. These experimental results suggest that TPS inactivates the enzyme phenylethanolamine-N-methyltransferase (PENMT), which converts NDR into ADR [11]. While TPS reduced adrenalin levels by suppressing this enzyme in intact rats, NDR and DOP levels was unaffected. The blood samples for measuring NDR and DOP levels were collected only 5 min after administering TPS, and this may be reason of why the plasma NDR level was not affected by TPS when the enzyme was suppressed. We could not find any reports about the short-term effect of adrenalindecreasing causing changes in theNDR and DOP levels. However there is information in the literature about the long-term effect of low adrenalin levelson increasing NDR and DOP levels more than 8 days from adrenelectomy [1]. This indicates that the increases g of NDR and DOP levels are due to the central nervous system. As is known; NOR and DOP are produced in adrenal medulla and central nervous system but thatadrenalin is produced only in adrenal medulla [4]. Our experimental results clearly show that it is necessary for the NDR and DOP levels to be affected by inhibition of the TPS-dependent enzymeand that PENMT should be inhibited for a long time or the level of adrenaline should be maintained longer. In adrenalectomized rats, NDR and DOP levels exhibited a significant increase compared with the levels in the intact rats, A previous study suggested that the reason for this increase was a responsedeveloped to fill the adrenaline gap in the body [4].

The results of the present study suggest thatthe plasma NDR level was not affected by ADR administration. Adrenalectomized rats were allowed to stand for 7 days after the surgical procedure and were used fort he experiment on the 8th day. Administration of single dose of epinephrine may have been insufficient to return the NDR level to normal after this 7-days period.

TPS induced hyperalgesia by reducing endogenous ADR production. Administration of TPS did not alter the level of ADR applied from the outside. TPS had no effect on endogenous NDR and DOP production. TPS needs to be administered together with ADR in order to establish analgesic activity in general anesthesia.

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Conflicts of Interests

The authors have no conflicts of interest.

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