PHYSIOLOGY

Delta Sleep-Inducing Peptide and Flunitrazepam Acting Jointly Increase Cardiac Electrical Stability

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In experiments on rabbits under Nembutal anesthesia, intravenous injection of flunitrazepam in a single dose of 0.125 mg/kg in combination with delta sleep-inducing peptide in a single dose of 60 nmol/kg raised the threshold of ventricular fibrillation to a level that was significantly higher than that after injection of flunitrazepam alone in the same dose and comparable to that after injection of this benzodiazepine alone in twice as high a dose (0.25 mg/kg), but without the side effects (persistent hypotension and transient bradycardia) produced by the latter dose. It is concluded that the ability of jointly acting delta sleep-inducing peptide and flunitrazepam to increase the electrical stability of the heart may have practical relevance to the prevention of ventricular arrhythmias occurring under conditions of emotional stress and myocardial ischemia.

Key Words: *cardiac electrical stability; delta sleep-inducing peptide; flunitrazepam; drug interactions*

Cardiac arrhythmias arising during emotional stress, after myocardial infarction, and in other disease states may result in sudden death from ventricular fibrillation; one of the mechanisms whereby the latter develops is a decrease in cardiac electrical stability [5,12]. Studies of the ways in which cardiac electrical stability can be increased be means of endogenous peptides and pharmacological substances may therefore constitute a promising line of research into the prevention of cardiac arrhythmias.

Delta sleep-inducing peptide (DSIP) has been shown to increase the resistance of animals to, and prevent their death from, cardiovascular disturbances during emotional stress [4]. It has been reported to raise the threshold of ventricular arrhythmias in intact animals and to return cardiac electrical stability to normal and exert an antiarrhythmic action in animals exposed to stress [1,6].

Pharmacological substances capable of increasing myocardial electrical stability include a number of psychotropics of the benzodiazepine class with pronounced effects on the heart [14]. However, the use of such drugs in doses that substantially raise the ventricular fibrillation threshold is limited by their side effects such as hypotension, reduced cardiac output, and (in some cases) slowed heart rate [7,10]. Accordingly, studies have been undertaken to explore the potential of using these psychotropic agents in combination with substances that act synergistically with them, thereby augmenting their beneficial effects on the heart [14].

The aim of the present study was to measure the combined effect of DSIP and flunitrazepam on cardiac electrical stability in view of the need to

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reduce doses and eliminate side effects of this benzodiazepine.

MATERIALS AND METHODS

This study involved acute tests on a total of 30 male Chinchilla rabbits (body weight 2.0-2.5 kg) under Nembutal (40 mg/kg) anesthesia. Eight of the rabbits were injected with DSIP in a single dose of 60 nmol/kg, 15 with flunitrazepam in a single dose of 0.25 or 0.125 mg/kg, and 7 with DSIP at 60 nmol/kg followed 5-10 min later by flunitrazepam at 0.125 mg/kg. DSIP and flunitrazepam were administered intravenously into an auricular vein and their combined effects on cardiac electrical stability, heart rate, and blood pressure were compared with those produced by each of them separately.

Cardiac electrical stability was estimated in artificially ventilated rabbits from the threshold of ventricular fibrillation elicited by a series of electrical pulses delivered during the vulnerable phase of the cardiac cycle [9]. The electrocardiogram was recorded in the standard lead II. Arterial and left-ventricular blood pressures were measured with a Mingograf-82 cardiopolygraph. The combined and individual effects of DSIP and flunitrazepam were estimated at 10 min and then every 30 min postinjection. The DSIP dose (60 nmol/kg) was chosen on the basis of our own findings [1,6] and the flunitrazepam dose of 0.25 mg/kg on the basis of data reported by Vrana *et al.* [14]. Flunitrazepam (Rohypnol) solutions were prepared as instructed by the manufacturer (Hoffman-La Roche). The DSIP we used had been synthesized at the Shemyakin Institute of Bioorganic Chemistry and kindly donated by Member of the Russian Academy of Sciences V. T. Ivanov and Senior Research Worker I. I. Mikhaleva.

The results were subjected to statistical analysis using Student's *t* test to estimate the significance of differences.

RESULTS

In the group (n=8) injected with DSIP alone at 60 nmol/kg, the ventricular fibrillation threshold rose significantly from 11.2±1.6 mA before injection to 17.8±2.0 mA 10 min after it (p<0.05), i.e., by 59% (Fig. 1, *a*), and it continued to rise later, reaching 20.3±2.3 mA by minute 30 (81% rise; p<0.01), and remained at a high level throughout the observation period - 20.7±1.2 mA at 60 min (85% rise; p<0.01), 19.3±1.3 mA at 90 min (72%; p<0.01), and 18.6±1.6 mA at 120 min (66%; p<0.01). The heart rate (HR) and mean arterial pressure (MAP) showed little change from their preinjection levels (267±9.3 beats/min and 90.7±3.5 mm Hg) at all times when they were measured. DSIP thus increased cardiac electrical stability without significantly altering either the HR or MAP.

In the group (n=7) given flunitrazepam alone at 0.25 mg/kg, the ventricular fibrillation threshold rose from 12.3±1.3 mA to 20.8±2.3 mA 10 min postinjection (p<0.01), i.e., by 69% (Fig. 1, *b*) and to 24.0±2.9 mA by 30 min (95% increase; p<0.01), and it remained significantly elevated over the baseline level at 60, 90, and 120 min, at which times it equaled 21.7±2.3 mA (76% rise; p<0.01), 19.7±2.4 mA (60%; p<0.02), and 18.3±1.7 mA (49%; p<0.02). However, this effect of flunitrazepam was accompanied by a marked MAP fall from 92.8±1.9 mm Hg at baseline to 72.8±8.4 mm Hg at 10 min (p<0.05), and the hypotensive effect persisted throughout the observation period, with the MAP being significantly lowered to 74.6±6.6 mm Hg at 30 min (p<0.05), 75.6±7.6 at 60 min (p<0.05), 73.8±7.5 at 90 min (p<0.05), and 75.0±7.8 mm Hg at 120 min (p<0.05). No significant changes in the HR were observed except at 10 min postinjection, when it was decreased to 240.0±10.0 beats/min from the baseline level of 267.0±6.5 (p<0.05). Flunitrazepam in this dose thus increased cardiac electrical stability while producing a sustained hypotensive effect and causing transient bradycardia.

The group (n=8) injected with flunitrazepam alone in the dose half that given to the preceding group (0.125 mg/kg) also showed significant but less marked increases in the ventricular fibrillation threshold (Fig. 1, *c*). The threshold rose from 11.6±1.2 mA at baseline to 16.0±1.5 mA at 10 min (p<0.02), i.e., by 48%, and to 18.7±1.7 mA at 30 min (61%; p<0.01), and it was still above baseline both at 60 min (17.3±1.9 mA, or 49% above; p<0.05) and at 90 min (17.0±1.1 mA, or 47% above; p<0.01). The MAP did not significantly change from its baseline level of 92.7±4.4 mm Hg during the observation period, nor did the HR, except by min 10, when it had dropped to 246.0±5.1 beats/min from the baseline value of 268.3±5.3. The lower flunitrazepam dose was therefore less effective in increasing cardiac electrical stability and, unlike the higher dose, did not produce a hypotensive effect.

In the group (n=7) administered flunitrazepam at 0.125 mg/kg 5-10 min after DSIP (60 nmol/kg), the ventricular fibrillation threshold was significantly higher than in the preceding group given flunitrazepam alone in the same dose (Fig. 1, cf.

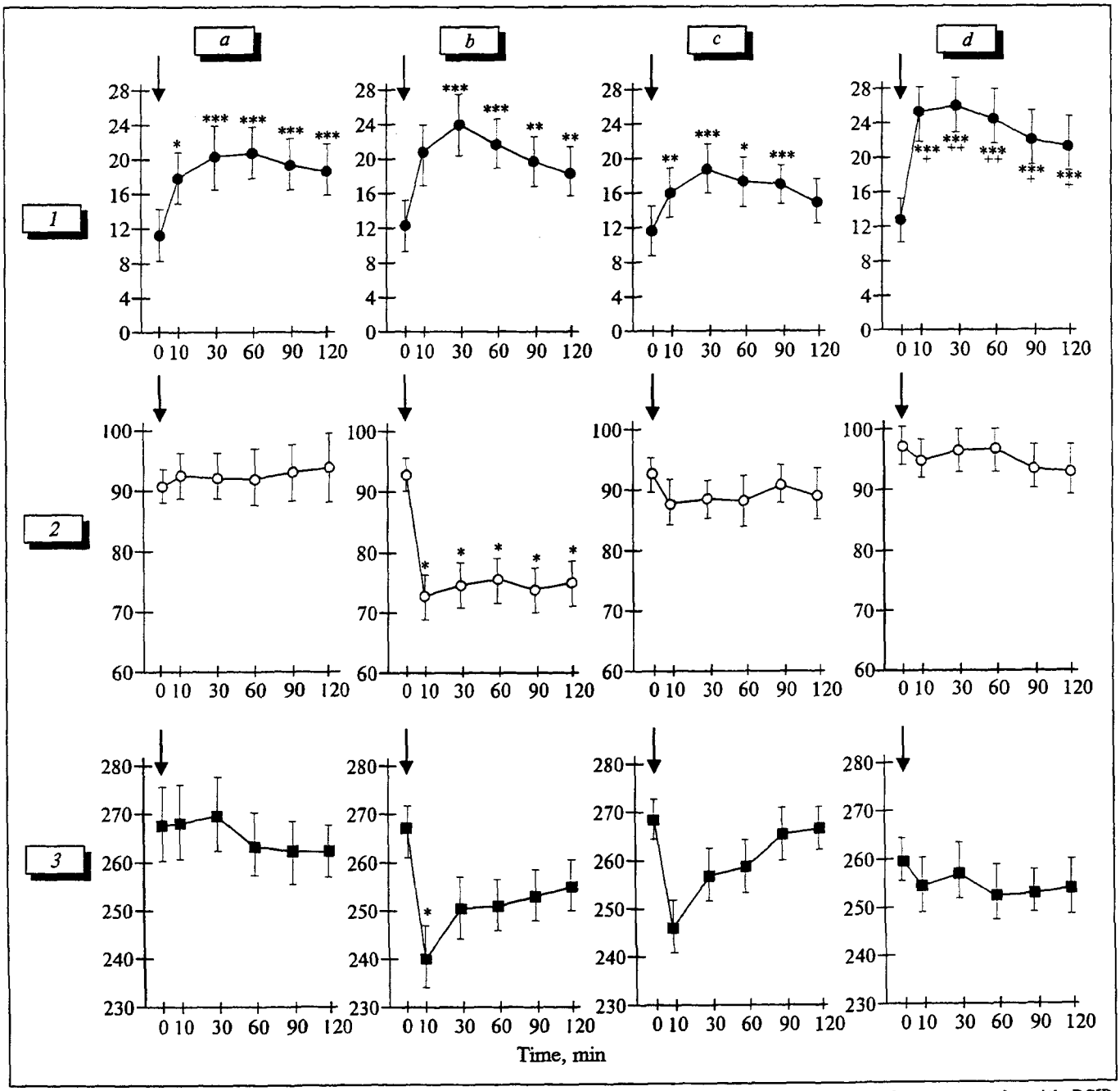

Fig. 1. Temporal variations in the ventricular fibrillation threshold, MAP, and HR in rabbits injected intravenously with DSIP, flunitrazepam, or both these compounds ($M \pm m$). *a*) DSIP 60 nmol/kg; *b*) flunitrazepam 0.25 mg/kg; *c*) flunitrazepam 0.125 mg/kg; *d*) flunitrazepam 0.125 mg/kg after DSIP (60 nmol/kg). *1*) ventricular fibrillation threshold, mA; *2*) MAP, mm Hg; *3*) HR, beats/min. The arrow marks the injection time. $^{*}p<0.05$, $^{**}p<0.02$, $^{***}p<0.01$ in comparison with the respective initial values. $^{+}p<0.05$, $^{++}p<0.02$ in comparison with values for the respective time intervals in *c* and *d*.

d against *c*). At 10 min after flunitrazepam injection in the presence of DSIP, the threshold equaled 25.3±1.4 mA compared with the baseline value of 12.8±0.9 mA, i.e., it increased by 98% ($p<0.01$), and it reached the highest level of 26.0±1.7 mA by 30 min (102% increase; $p<0.01$) to remain at significantly elevated levels at 60 min (24.5±2.0 mA, or 91% above baseline; $p<0.01$), 90 min (22.1±2.3 mA, or 72%; $p<0.01$), and 120 min (21.3±2.3 mA, or 66%; $p<0.01$). Neither the MAP nor the HR were observed to change appreciably from the baseline values of 97.2±2.6 mm Hg and 259.6±9.8 beats/min throughout the two-hour observation period.

The detailed data presented above clearly show that DSIP and flunitrazepam acting jointly were more effective in increasing cardiac electrical stability than flunitrazepam alone in the same dose

(0.125 mg/kg); moreover, they did not alter either the MAP or the HR. Comparison of the group given flunitrazepam alone in a dose twice as high (0.25 mg/kg) with that given both DSIP and flunitrazepam at 0.125 mg/kg indicates that while the ventricular fibrillation threshold was increased to similar degrees in these groups, the side effects characteristic of this benzodiazepine (persistent hypotension and transient bradycardia) were absent in the latter group.

The use of benzodiazepines as antiarrhythmic agents is predicated on the concept that activation of the sympathoadrenal system plays a pathogenic role in the production of spontaneous ventricular fibrillation during emotional stress and after myocardial infarction [2,5,12]. In the present study, flunitrazepam in a dose of 0.25 mg/kg significantly raised the ventricular fibrillation threshold in intact rabbits. A similar effect of this drug was observed in a dog model of acute myocardial infarction [14]. In our experiments the threshold-raising effect of flunitrazepam at this dose level was accompanied by changes in MAP and HR. Certain benzodiazepines, including flunitrazepam, that mainly act by dilating peripheral vessels may also decrease cardiac output, lower systolic and diastolic blood pressure, and cause bradycardia [7,10]. When its dose was decreased by half, flunitrazepam did not elicit adverse reactions but the increase it caused in cardiac electrical stability was less pronounced.

Since DSIP had been shown to raise the threshold of ventricular arrhythmias and to prolong cardiac effects of the vagus nerves while suppressing those of sympathetic nerves [6], we used DSIP as a possible benzodiazepine synergist, and administered a lower flunitrazepam dose in its presence. We found that this peptide potentiated the beneficial effect of flunitrazepam, with no side effects being observed.

The combined effect from jointly acting DSIP and flunitrazepam is probably a result of their interaction involving the GABA-ergic system, which plays an important part in the regulation of cardiovascular functions [2,8]. The major effects of benzodiazepines are known to be determined by their influences on GABA-ergic processes [13]. DSIP, for its part, influences the level and metabolism of GABA [3]. Moreover, although there is no evidence of direct DSIP involvement in the specific binding of benzodiazepines to their receptors, this peptide can activate GABA binding, thereby manifesting benzodiazepine-like activity [11].

The possibility of potentiating benzodiazepine action on cardiac electrical stability has been demonstrated on a dog model of acute myocardial infarction using several substances of the benzodiazepine class in combination with potent analgesics [14]. The use of such combinations greatly mitigated the side effects characteristically produced by the benzodiazepines and analgesics.

The enhancement of cardiac electrical stability seen in our study with the combined use of DSIP, which is an endogenous stress-relieving factor, and flunitrazepam, which has found extensive clinical application, may help prevent ventricular arrhythmias under conditions of emotional overstrain or myocardial ischemia.

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