Psychomotor Performance and Counterregulatory Responses During Mild Hypoglycemia in Healthy Volunteers

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The effect of mild hypoglycemia on psychomotor performance and counterregulatory responses was studied among 12 healthy volunteers. Each volunteer received two modified hyperinsulinemic glucose clamps. One morning, plasma glucose was held constant at euglycemic levels (4.9 mM) for 95 min, and another morning, it was lowered over 35 min and then held constant at hypoglycemic levels (3.4 mM) for 60 min. A battery of psychomotor tests and a questionnaire assessing hypoglycemic symptoms were administered before and repeated during the last 30 min of each clamp. The questionnaire and three selected psychomotor tests were also administered repetitively during the 1st h of each clamp. During the hypoglycemic studies, a rise was seen in plasma epinephrine and pancreatic polypeptide at 45 min. An increase in symptom scores was first recorded at 50 min during the hypoglycemic studies [median 4 (range 0–13) vs. 2 (5–6), $P < .05$]. Performance was impaired on two psychomotor tests included in the battery. One was the trail making test on fine motor performance (-19.3 ± 4.2 targets/min, mean ± SE vs. 1.2 ± 4.8 targets/min, $P < .05$), and the other was the digit-symbol substitution (DSS) test on information processing and memory (18 ± 3 vs. 29 ± 4 symbols/min, $P < .03$). Of the tests administered during the 1st h, performance was impaired on the DSS. This impairment became significant at 45 min (14 ± 4 vs. 22 ± 4 symbols/min, $P < .005$). In conclusion, mild hypoglycemia selectively impairs psychomotor performance in healthy volunteers but not before the onset of glucose counterregulation and warning symptoms. *Diabetes Care* **12:12–17, 1989**

Glucose	1 mM	= 18 mg/dl
Glucagon	1 ng/L	= 1 pg/dl
Epinephrine	1 pM	= 0.183 pg/ml or ng/L
Norepinephrine	1 nM	= 169 pg/ml or ng/L
Pancreatic polypeptide	1 pM	= 4.18 pg/ml or ng/L
Insulin	1 pM	= 0.014 μU/ml or mU/L

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The clinical effects of hypoglycemia can be divided into two broad categories: those that result from activation of the autonomic nervous system and those caused directly by neuroglycopenia (1). Evidence exists that glucose counterregulatory mechanisms, including those of the autonomic nervous system, are stimulated by decrements of plasma glucose to concentrations that lie within or just below the physiological range (2–4). Neuroglycopenia may develop at similar plasma glucose levels; electroencephalogram (EEG) studies have demonstrated changes in cortical activity at blood glucose concentrations in the range 53–85 mg/dl (2.9–4.7 mM) (5). Additional evidence shows that altered behavioral performance also occurs at similar levels of plasma glucose (6–10). The plasma glucose threshold at which the symptoms associated with glucose counterregulation develop has also been investigated. Available evidence suggests that such symptoms may develop at a lower threshold than that which stimulates glucose counterregulatory mechanisms, including those of the autonomic nervous system (4).

In our study, measurements of hypoglycemic symptoms, behavioral performance, and counterregulatory hormones were made simultaneously under conditions of mild hypoglycemia. We wanted to determine whether abnormalities of behavioral performance occurred during mild hypoglycemia, and if they did, whether they

occurred before or coincided with the appearance of symptoms or biochemical evidence of activation of counterregulatory hormone responses. To answer these questions, healthy volunteers were studied under euglycemic and hypoglycemic conditions by means of a modified hyperinsulinemic glucose-clamp technique. Before and during the clamp periods, repeated measurements of counterregulatory hormones and hypoglycemic symptoms were made. A wide range of psychomotor tests were used to provide a detailed profile of behavioral performance under each test condition.

MATERIALS AND METHODS

Subjects. Twelve right-handed nondiabetic volunteers (mean age 23 yr, range 18–27; 6 men, 6 women) were recruited. They submitted informed written consent to participate in the study, which was approved by the ethical committee of the Queen's University Faculty of Medicine. Potential volunteers were excluded from the study if they had taken any medication within the preceding 2 wk. Women completed the study between days 3 and 14 of their menstrual cycle.

Before the study began, each volunteer took part in two practice sessions to allow familiarization with the psychomotor tests being used and to minimize subsequent practice effects during the study. The second practice session was a full mock-up of the study day, including insertion of an intravenous cannula. Volunteers were told that a prize of ~$35.00 would be awarded to the individual with the best aggregate score over both test periods.

Protocol. Each volunteer was studied on two separate mornings (3–7 days apart) when either fasting euglycemic or hypoglycemic (target 3.2 mM) conditions were maintained. A crossover design that was balanced for treatment order was used in which 6 volunteers received a euglycemic clamp first and the remaining 6 received a hypoglycemic clamp. The investigator responsible for the psychomotor testing and the volunteer were unaware of the treatment order.

Volunteers were checked in at 0830 after an overnight fast. They were asked to sleep their normal number of hours the night before, to abstain from alcohol for the 2 days before the study, and to avoid smoking on the morning of the study. On arrival, the volunteers rested while an intravenous (18-gauge) cannula was inserted into their dominant forearm for infusion of dextrose and insulin. A second intravenous cannula was placed retrogradely into their nondominant distal forearm for blood sampling; this arm was subsequently kept heated at 50°C to achieve arterialization of venous blood. During the study period the volunteer was seated.

At −45 min, baseline blood samples for plasma glucose, insulin, glucagon, pancreatic polypeptide, epinephrine, and norepinephrine were taken. An infusion of normal saline (0.5 ml/min) was then started, and baseline measurements of symptoms and psychomotor performance were made. At 0 min, a constant infusion of human soluble insulin (Humulin S, Lilly, Indianapolis, IN) at a rate of 1 mU · kg^{-1} · min^{-1} was started and maintained for 95 min. To maintain euglycemic conditions, a variable infusion of 20% dextrose was initiated 5 min later. For hypoglycemic studies, the dextrose infusion was delayed until the target plasma glucose level was reached. The infusion rate of dextrose was adjusted according to a clamping algorithm (Oxclamp, D. Matthews, Oxford, UK) to maintain plasma glucose at the desired level.

Laboratory analysis. Plasma glucose measurements were taken every 5 min with a Beckman glucose analyzer II (Fullerton, CA). The calibration of this machine was checked at 20-min intervals by means of a standard glucose solution and double-checked with a control serum.

Blood was drawn for hormone measurements at 15-min intervals. Plasma epinephrine and norepinephrine levels were determined by high-performance liquid chromatography (11). Insulin, glucagon, and pancreatic polypeptide were measured by radioimmunoassay (12).

Hypoglycemic symptoms. Symptoms of hypoglycemia were measured by questionnaire at 0, 10, 30, 50, and 90 min. Eleven symptoms including sweating, trembling, warmness, pounding heart, hunger, tiredness, weakness, sleepiness, confusion, queasy stomach, and apprehension were individually rated as being absent, mild, moderate, or severe. These ratings were scored as 0, 1, 2, and 3, respectively. The sum of the 11 individual rating scores from each questionnaire provided a total symptom score for each observation time.

Psychomotor tests. A battery of psychomotor tests that took 30 min to complete was administered twice, first before each clamp started, to provide a baseline, and then between 60 and 95 min, to provide test results. An automated psychological testing system, designed by Elithorn et al. (13) was used to administer two simple reaction-time tests (1 auditory and 1 visual), a finger-tapping test and a trail making test. Trail making as used in this system required the volunteer to move a cursor around a visual display unit screen by means of a joystick, hitting a sequence of numbered and lettered targets. A Leeds psychomotor test apparatus was used to test choice reaction time and to measure critical flicker-fusion threshold (14). One task requiring pencil and paper, the digit-symbol substitution (DSS) test, was also included in the battery (15).

Three of these tests, choice reaction time, DSS, and critical flicker-fusion threshold, were administered repetitively at 10-min intervals during the 1st h of each study. Adequate practice time was given on all psychomotor tests, to allow for familiarization. Practice effects were minimized on all tests except DSS. In this test, volunteers were provided with a series of nine symbols coded 1–9. Then they were presented with a sheet of uncoded symbols and asked to write the appropriate numerical code below each symbol. They were given 1 min to complete as many as possible. The code was

changed for each study day, and a learning curve was plotted for each of these days.

Statistical analysis. Results for plasma glucose and psychomotor tests are expressed as means ± SE. Analysis of these results was by paired *t* test via the method of Hills and Armitage for crossover design studies (16). The results for hormone analysis are not normally distributed and are expressed as median values with ranges. Analysis of these results was by Wilcoxon's paired-rank test. The questionnaire scores expressed on an ordinal scale are medians with ranges and were analyzed by the Hills and Armitage method (16).

RESULTS

Plasma glucose and insulin. The initial fasting plasma glucose level of both euglycemic and hypoglycemic studies was 5.0 ± 0.1 mM (Fig. 1). The plasma glucose concentration maintained during the euglycemic battery-test period (60–90 min) was 4.9 ± 0.0 mM. During the hypoglycemic studies, the plasma glucose fell to 3.2 ± 0.1 mM at 35 min and was 3.4 ± 0.1 mM for the battery-test period. Stable hyperinsulinemic conditions were achieved within 15 min of starting the insulin infusion, and plasma insulin concentrations were similar for both study periods.

Glucagon, epinephrine, pancreatic polypeptide, and norepinephrine. Significant increases in glucagon, epinephrine, and pancreatic polypeptide occurred during hypoglycemic conditions, when compared with euglycemic conditions (Figs. 2 and 3). Glucagon concentrations were significantly higher at 30 min [median 145 ng/L (range 105–250 ng/L) vs. 105 ng/L (80–145 ng/L), $P < .01$], but significant increases in epinephrine [288 ng/L (70–1480 ng/L) vs. 31 ng/L (0–144 ng/L), $P < .01$] and pancreatic polypeptide [138 ng/L (25–1080 ng/L) vs. 33 ng/L (20–50 ng/L), $P < .01$] did not occur until 45 min. There was no difference in norepinephrine levels between the two glycemic conditions.

Symptoms. There was no increase in total symptom score between euglycemic and hypoglycemic levels at 10 and 30 min, but scores at 50 and 90 min were signficantly higher under hypoglycemic conditions compared with euglycemic conditions (Table 1).

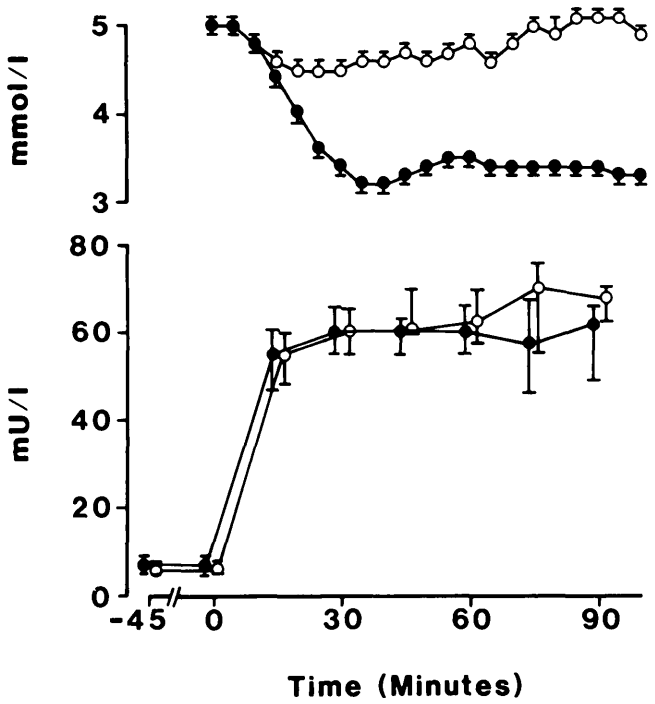

FIG. 1. Plasma glucose (means ± SE; *top*) and plasma insulin (median ± range from 1st to 3rd quartile; *bottom*) concentrations during euglycemic (○) and hypoglycemic (●) study periods. Conversion factor to SI units for insulin is 7.18 pM.

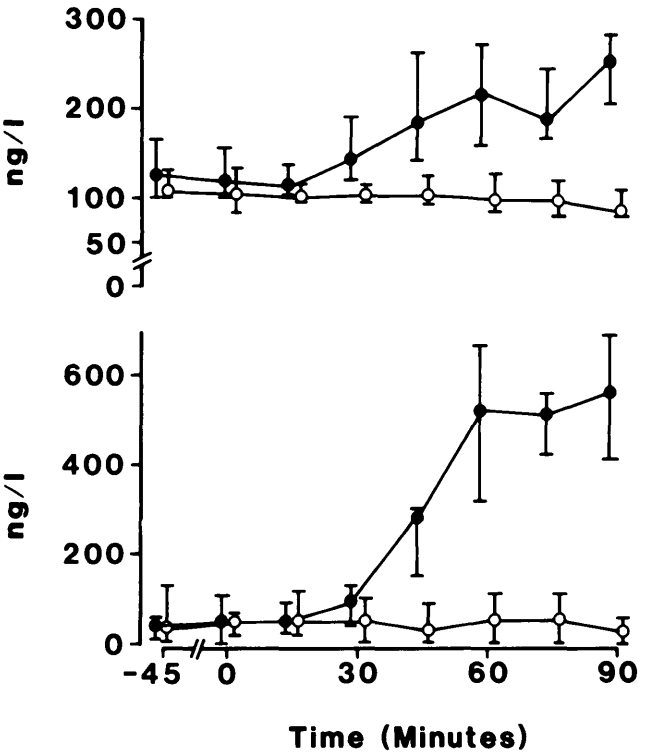

FIG. 2. Plasma glucagon (median ± range from 1st to 3rd quartile; *top*) and plasma epinephrine (*bottom*) concentrations during euglycemic (○) and hypoglycemic (●) study periods. Conversion factor to SI units for epinephrine is 5.46 pM.

TABLE 1
Symptom scores

Time (min)	Hypoglycemia	Euglycemia	*P*
10	1 (−4, 5)	1 (−3, 3)	NS
30	1 (−4, 5)	2 (−3, 4)	NS
50	4 (0, 13)	2 (−5, 6)	<.05
90	3 (−1, 11)	1 (−4, 7)	<.01

Results expressed as change from baseline (median with range in parentheses). NS, not significant.

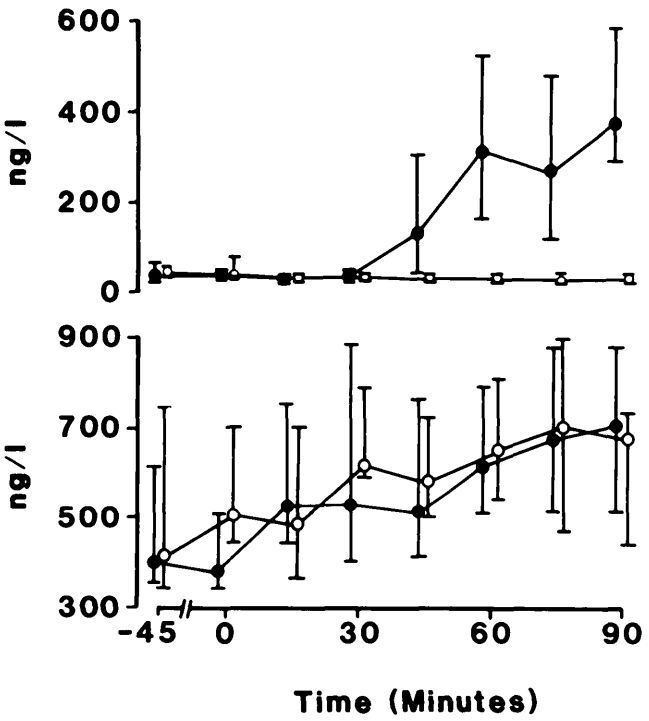

FIG. 3. Plasma pancreatic polypeptide (median ± range from 1st to 3rd quartiles; *top*) and plasma norepinephrine (*bottom*) concentrations during euglycemic (○) and hypoglycemic (●) study periods. Conversion factor to SI units for pancreatic polypeptide is 0.239 pM and for norepinephrine is 0.026 mM.

Psychomotor tests. The results for the battery-test period are shown in Table 2. Two tests demonstrated significantly impaired performance during hypoglycemia: trail making and DSS. Of the three tests administered repetitively during the 1st h, only the performance on DSS was significantly impaired during hypoglycemia (Fig. 4).

DISCUSSION

Our data demonstrate that in healthy volunteers a small decrement in plasma glucose, close to the physiological postabsorptive range, results in selective impairment of psychomotor performance. The same decrement also initiated glucose counterregulation with the release of glucagon and epinephrine and stimulation of the parasympathetic nervous system, as evidenced by pancreatic polypeptide release. Furthermore, this counterregulatory response coincided with the development of symptoms of hypoglycemia.

When using psychomotor test techniques to study behavior, many variables can affect the results. These include the age, sex, personality, academic achievement, test strategy, and motivation of the volunteers. One way to control for these variables is by use of a crossover design, preferably double blind, in which treatment order is counterbalanced. Other variables that must be taken into account are time of day (as psychological function may show diurnal rhythm); environmental factors, such as heat and light; and practice effects (17,18). The tests themselves measure different aspects of behavior and vary in their sensitivity to different stresses. The effects of mild hypoglycemia on psychomotor performance are not firmly established, therefore it is wise to use a variety of tests rather than depend on only one. Of the battery of psychomotor tests administered in this study, performance deteriorated in two: trail making, a test on fine coordinated movement with little central information processing, and DSS, which is primarily a cognitive test on information processing but when administered repetitively also tests memory (19). The results for DSS are consistent with the findings of Holmes et al. (6), who demonstrated in insulin-dependent diabetes mellitus (IDDM) subjects a slower rate of remembering information as tested by a simple calculation test. A later study by Holmes et al. (7) on the effects of hypoglycemia on verbal fluency and naming performance also supported our findings, because these tasks involved information processing. A trail-making test was included in the study by Pramming et al. (8) on IDDM patients. They found a significant impairment of performance when plasma glucose levels were 1.8 mM but not at 2.9 mM. This inconsistency with our results may have been due to methodological differences. Similarly, Ipp and Forester (20) found performance on a trail-making test by normal volunteers to be impaired by severe but not mild hypoglycemia. In Ipp's study, however, one may speculate that small subject numbers and a documented practice effect may have masked an early impairment of performance.

Simple motor responses, as tested by finger tapping,

TABLE 2
Battery-test results

Psychomotor tests	Euglycemia	Hypoglycemia	*P*
Finger tapping (taps/s)	0.4 ± 0.1	0.5 ± 0.1	NS
Auditory reaction time (s)	0.015 ± 0.035	0.027 ± 0.049	NS
Visual reaction time (s)	0.023 ± 0.030	0.011 ± 0.052	NS
Choice reaction time (s)	0.001 ± 0.020	0.029 ± 0.008	NS
Critical flicker fusions (Hz)	−0.7 ± 0.5	−1.6 ± 0.4	NS
Trail making (targets/min)	1.2 ± 4.8	−19.3 ± 4.2	<.005
Digit-symbol substitution (symbols/min)	29 ± 4	18 ± 3	<.003

Scores expressed as change from baseline (means ± SE). NS, not significant.

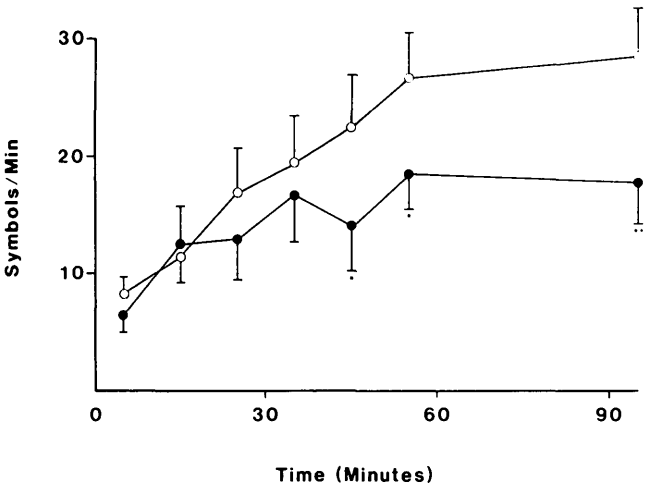

FIG. 4. Digit-symbol substitution test scores (means ± SE) during euglyemic (○) and hypoglycemic (●) study periods. *$P < .005$, **$P < .003$.

have been shown by Holmes et al. (9), Pramming et al. (8), and this study to be unaffected by mild hypoglycemia. Reaction times that require a motor response to some sensory stimulus have also been extensively studied. Holmes showed that simple tests requiring a single response to a single stimulus are relatively unaffected by mild hypoglycemia. However, more complex reaction-time tests, where a choice of response is required and where decision making and cognition are introduced, were found by Holmes to be sensitive to mild hypoglycemia (9). Our results for simple reaction time agreed with those of Holmes, but on the choice reaction time we did not demonstrate any impairment during hypoglycemia. Exact comparisons of the two studies are difficult because Holmes' study was on diabetic patients who were subjected to considerably longer periods of hypoglycemia (up to 3 h). The duration of hypoglycemia in the development of neuroglycopenia and altered psychomotor function has not been established, but it is possible that psychomotor function may become increasingly impaired with prolonged mild hypoglycemia. Evidence that supports this comes from our results for the DSS test. Figure 4 shows that the difference in performance between euglycemic and hypoglycemic periods increased with time. Reaction time has also been studied by Heller et al. (10). By use of a serial reaction-time task they demonstrated impairment of performance at plasma glucose concentrations of 3.2 mM in both diabetic and nondiabetic subjects (21). This test was administered repetitively for 5 min, requiring vigilance to maintain sensory motor responsiveness. The early impairment of performance noted by Heller may therefore be due to the inability to sustain vigilance.

Critical flicker-fusion threshold, which may be defined as the point at which a flickering light gives rise to the subjective sensation of a steady light, is considered to be a test of arousal having been found sensitive to the effects of sedative and stimulant drugs (19,22). We found that thresholds fell in conjuction with lower arousal levels during both hypoglycemic and euglycemic clamps, although the test did not discriminate between the two conditions. The DSS test as we used it, showed a significant impairment at the same time as the automatic nervous system was activated and related symptoms developed. It is not possible from our data to say whether neuroglycopenia or the side effects of counterregulation were responsible for the impairment of psychomotor performance. Analysis of the learning curves in Fig. 4 suggests a trend toward impaired performance at 30 min and before the activation of the autonomic nervous system. Heller et al. (10) demonstrated impairment of performance on serial reaction time at 3.2 mM before awareness of hypoglycemia developed, but interestingly not before the onset of an epinephrine response. Herold et al. (23) demonstrated that the effect of hypoglycemia on a reaction-time task was independent of symptoms due to hypoglycemia. In the same study, the magnitude of the counterregulatory hormone response did not correlate with the change in reaction time. Pramming et al. (8) observed an early decrement in psychomotor performance before a significant increase in hypoglycemic symptoms developed. These data suggest that the observed impairment of psychomotor performance during hypoglycemia is not an effect of the unpleasant symptoms and associated ill feeling. The effect on psychomotor performance of rises in counterregulatory hormones and associated symptoms does, however, require further investigation by means of an appropriately designed study.

Our findings demonstrate that symptoms of hypoglycemia do not occur immediately after the onset of hypoglycemia but develop later, sometime after the initial activation of the autonomic nervous system. This may provide part of the explanation for the findings by others that diabetic subjects may incorrectly estimate their plasma glucose or be unaware of hypoglycemia under experimental glucose-clamp conditions (24). This study observed in detail the effects of mild hypoglycemia on psychomotor performance in healthy volunteers. It confirms the usefulness of selecting various psychomotor tests as tools for monitoring behavioral activity and cerebral function under such conditions. The investigation of nondiabetic subjects in this context is important because it helps to define safe lower limits for plasma glucose concentration. Comparison with similar studies that have used diabetic patients are difficult because such patients may have impaired counterregulatory responses and altered thresholds for the onset of counterregulation and neuroglycopenia (25,26). There is a need for a single study to make a detailed comparison of psychomotor performance in diabetic and nondiabetic subjects during hypoglycemia.

In conclusion, we found that mild hypoglycemia impairs psychomotor performance on tests requiring fine motor performance, memory, and information processing skills in healthy volunteers. A significant impairment of performance, however, was not detected before the

onset of glucose counterregulation and warning symptoms.

ACKNOWLEDGMENTS

We acknowledge Professor K.D. Buchanan and W. Leahy for providing technical help and hormone assays and C. Patterson for providing statistical advice.

A.B.S. was the recipient of a Department of Health and Social Services Research Fellowship.

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