Table 1. Quantitative estimation (μg/ml) of acetic acid and octanoic acid in MF of three tigers. Six replications of tigers 1 and 3 and four replications of tiger 2 were made

	Acetic acid	Octanoic acid
Tiger 1	3.9	16.5
	2.4	50.4
	5.0	10.4
	0.8	34.3
	6.2	25.4
	0.2	22.7
Tiger 2	26.2	11.7
	16.4	12.5
	14.0	6.1
	15.0	1.4
Tiger 3	9.9	15.9
	4.7	19.3
	3.1	51.4
	8.3	46.2
	9.0	14.0
	5.6	21.2

further confirmed the identity of these FFA.

Six, four and six replications of the quantitative values (μg/ml) of FFA in the MF of tigers 1, 2 and 3, collected over a long period of time, show a wide range of variation.

This situation is very different from that of the mongoose[1]. The values for Euclidean distance and Mahalanobis distance between any pair of the three sets of values for the tigers have been calculated by taking average values, but in view of the wide range of variation we have chosen another method of representing the genetic distance of the three tigers. Figure 1 shows a method of visually representing this. Each polygon encloses the quantitative values of FFA in the three tigers (6, 4 and 6 for tigers 1, 2, and 3 respectively). For acetic acid the two polygons 1 and 3 are apparently close together while 2 seems to be a more distant set. For octanoic acid this trend is still recognizable while for the nine other acids the polygons overlap. If one or two FFA (like acetic acid) differ significantly in the three tigers, these values as well as the ensemble of ratios of all the FFA (which will necessarily be different in the three tigers) can serve as the basis for individual distinction. Likewise the ratios and proportions of the other volatiles such as amine, aldehyde, etc. present in MF can also play a role.

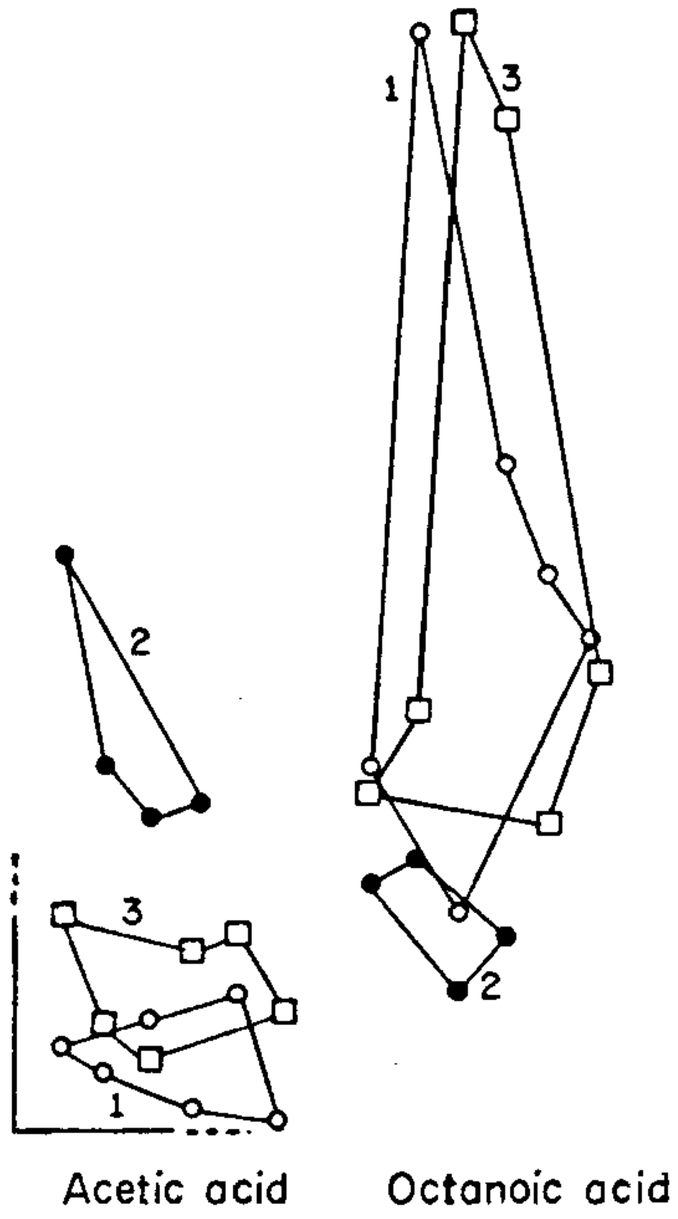

Figure 1. X-axis (vertical) represents μg/ml; Y indicates no. of samples for each of the three tigers 1, 2 and 3.

A search for the pedigree of the three tigers as learnt from the zoo staff at Nandan Kanan, Orissa and confirmed by consulting the stud book[5] reveals that tigers 1 and 3 are mother and son respectively while tiger 2, a female, is distantly related to tiger 3. The polygons in Figure 1 do suggest that 1 and 3 are closer to each other than to 2. Stud book numbers of tigers 1, 2, 3 are 186, 358 and 363, respectively.

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The antiepileptic drug sodium valproate affects body weight in *Drosophila*

Drosophila gene *Shaker* (*Sh*) encodes a voltage-sensitive K^+ channel[1]. *Sh*-like K^+ channels are conserved from bacteria to man[2,3]. Recently, mutations in a gene encoding one such channel has been found to be associated with human epilepsy[4,5]. Sodium valproate (NaVP) is an antiepileptic drug that possibly acts through voltage- and use-dependent blockade of Na^+ channel[6–8]. It is also likely to have other activities such as reduction of voltage-dependent Ca^{++} current and GABA-mediated inhibition. A unique side effect of NaVP in human epileptics is weight gain due to appetite stimulation[6,9]. We report here that treatment of Oregon-R (OR) and Canton-S (CS) wild-type, and Sh^3, Sh^5, Sh^6 and Sh^{14} mutant *Drosophila melanogaster* flies with NaVP results in body weight loss in all cases except Sh^5. This

allele-specific resistance to weight reduction might suggest a role of *Sh* K^+ channel in body weight regulation. We further show that, like Sh^5, $para^{ts1}$, a temperature-sensitive Na^+ channel mutant[10], is also resistant to NaVP-induced weight loss. This indicates that NaVP acts through *para* Na^+ channel, supports the hypothesis that NaVP has voltage- and use-dependent Na^+ channel blockade activity and, in turn, highlights the structural and functional conservation of Na^+ channel between fly and man. We also report that NaVP causes increased mortality in OR, CS, Sh^3, Sh^6, Sh^{14} and $para^{ts1}$, compared to Sh^5. As both Sh^5 and $para^{ts1}$ are resistant to NaVP-induced body weight loss, the decreased drug-induced mortality in only Sh^5, not $para^{ts1}$, indicates that loss of body weight is not directly responsible for death of flies. By recording NaVP-induced mortality in Sh^5/OR and Sh^5/CS F_1 individuals, we show that the observed resistance to NaVP-induced lethality in Sh^5 is a dominant character.

Compared to normal food, weight gain in female flies fed on food containing NaVP showed a significant reduction in OR ($P < 0.001$), CS ($P < 0.01$), Sh^3 ($P < 0.001$), and Sh^{14} ($P < 0.001$) (Table 1). Sh^5 females, however, did not show any significant difference ($P > 0.01$) between the two body weights. Similarly, males registered a significant loss of body weight in OR ($P < 0.001$), CS ($P < 0.01$), Sh^3 ($P < 0.001$), Sh^6 ($P < 0.05$) and Sh^{14} ($P < 0.05$), whereas Sh^5 and $para^{ts1}$ males did not show any significant difference ($P > 0.1$) between the body weights of flies fed on normal and NaVP food (Table 1). To understand if NaVP-induced differences in weights of the flies indicate a drug-specific response, we treated OR and Sh^5 flies with another antiepileptic drug phenytoin sodium (PHT), under conditions similar to those used for NaVP (Table 1). The mean ± S.E.M. of percent increase in body weights of PHT-treated female flies, compared to flies fed on normal food, were observed to be 0.87 ± 0.02 and 0.72 ± 0.04, respectively. PHT was therefore not found to have, unlike NaVP, different effects on body weights of OR and Sh^5 flies ($P > 0.05$). This suggested the observed differences in the body weights of NaVP-treated flies to be drug-specific. To understand if different effects of NaVP on body weights of flies are due simply to differences in their feeding behaviour (taste- and/or smell-avoidance), we kept starved OR and Sh^5 flies in vials containing either normal or NaVP containing food and observed the feeding behaviour in the flies for some time immediately after they were transferred to food vials. Although NaVP-mixed food attracted relatively less number of both OR and Sh^5 flies than normal food, no strain-specific difference was observed in their feeding behaviour (data not presented). This suggested that it is, at least in part, the physiology, not simply the behaviour of flies that is affected by NaVP. In brief, weight loss experiment showed that NaVP reduced body weight in OR, CS, Sh^3, Sh^6 and Sh^{14}, not Sh^5 and $para^{ts1}$.

With respect to mortality, the following results were obtained. NaVP treatment caused increased mortality in OR, CS, Sh^3, Sh^6, Sh^{14} and $para^{ts1}$, compared to Sh^5 (Table 2). Both Sh^5 male and female flies were relatively resistant to the drug-induced mortality. The T(half) of OR, CS, Sh^3 and Sh^{14} female flies treated with NaVP differed from that of Sh^5 in a highly significant manner ($P < 0.001$; Table 2). It is also apparent from Table 2 that the difference between T(half) of OR, CS, Sh^3, Sh^6, Sh^{14} and $para^{ts1}$ male flies treated with NaVP and that of Sh^5 is highly significant. A similar comparison of T(half) of both male and female flies subjected to continued starvation (no food, Table 2) showed no difference ($P > 0.05$), except in case of Sh^{14} males whose T(half) differed from that of Sh^5 males ($P < 0.05$). 'No food' control was carried out to understand whether different mortality rates resulting after NaVP treatment is due simply to differences in their tolerance to starvation or to actual differences in their susceptibility to NaVP-induced toxicity. The former situation might be expected to arise due to differences in the feeding behaviour of the flies with respect to NaVP-mixed food (taste- and/or smell-avoidance). If reduced mortality in Sh^5 had resulted from an increased tolerance of these flies to starvation, Sh^5 should have been found to be more tolerant to starvation than other flies. This was however not found to be the case. 'No food' control data (Table 2)

Table 1. Mean ± S.E.M. of increase in body weight per fly (in mg), after treating starved flies with either normal food or food containing NaVP

	Female		Male	
	Normal food	NaVP food	Normal food	NaVP food
OR	0.32 ± 0.03 (7)	0.14 ± 0.02 (7)	0.17 ± 0.005 (3)	0.09 ± 0.002 (3)
CS	0.31 ± 0.04 (6)	0.11 ± 0.03 (6)	0.15 ± 0.004 (2)	0.11 ± 0.004 (2)
Sh^3	0.31 ± 0.02 (6)	0.16 ± 0.01 (6)	0.16 ± 0.002 (3)	0.10 ± 0.005 (3)
Sh^5	0.33 ± 0.06 (6)	0.32 ± 0.05 (6)	0.15 ± 0.005 (3)	0.16 ± 0.005 (3)
Sh^6	*	*	0.16 ± 0.02 (2)	0.06 ± 0.01 (2)
Sh^{14}	0.25 ± 0.02 (6)	0.10 ± 0.02 (6)	0.17 ± 0.004 (2)	0.13 ± 0.007 (2)
$para^{ts1}$	*	*	0.13 ± 0.01 (2)	0.12 ± 0.01 (2)

Cultures were grown on a standard *Drosophila* medium containing maize powder, sugar, yeast and Nipagin. Flies were raised and further treated at 20 ± 2°C. Conditions of culture and subsequent treatments were kept identical for all the strains. Standard fly manipulation methods were followed. Up to 3–4 day old flies were first starved for 20–22 h and then batches of 25–30 individuals were shifted to empty vials. Subsequently, flies in each vial were weighed in groups, before being shifted to vials containing either normal food or food containing 2 mg/ml NaVP (Sanofi-torrent). Flies in each vial were again weighed in groups after 18–20 h of feeding. Numbers in parentheses indicate the number of vials examined. *indicates non-availability of mutant females in the compound–X chromosome bearing stocks used, C(1) DX *y w f*/Sh^6 and C(1) DX *y f*/$para^{ts1}$.

Table 2. Mean ± S.E.M. of *T*(half), the time (in h) at which half of the total number of flies died

	Female		Male	
	No food	NaVP food	No food	NaVP food
OR	19.62 ± 2.37 (8)	84.50 ± 4.06 (8)	13.33 ± 2.40 (3)	62.00 ± 5.29 (3)
CS	15.25 ± 0.81 (8)	77.50 ± 7.27 (8)	9.330 ± 0.66 (3)	75.66 ± 2.96 (3)
Sh^3	19.33 ± 2.90 (3)	75.66 ± 2.33 (3)	11.00 ± 1.00 (2)	71.50 ± 3.50 (2)
Sh^5	20.12 ± 2.68 (8)	150.2 ± 11.89 (8)	11.33 ± 0.66 (3)	#
Sh^6	*	*	13.50 ± 1.50 (2)	46.00 ± 2.00 (2)
Sh^{14}	19.33 ± 2.90 (3)	66.00 ± 3.05 (3)	17.50 ± 1.50 (2)	68.00 ± 4.00 (2)
Sh^5/OR	14.00 ± 2.00 (2)	170.0 ± 45.12 (2)	$	$
Sh^5/CS	14.00 ± 2.00 (2)	157.0 ± 13.03 (2)	$	$
$para^{ts1}$	*	*	15.00 ± 1.73 (3)	71.66 ± 15.91 (3)

Up to 3–4 day old flies were first starved for 20–22 h and then 25–30 individuals were shifted to either empty vials (no food) or vials containing NaVP food. Number of live/dead flies were counted every 10–14 h. Loss of flies due to handling was corrected for. Sh^5/OR, F_1 females resulted from a cross between Sh^5 males and OR females. Sh^5/CS, F_1 females resulted from a cross between Sh^5 males and CS females. # indicates that less than one half of the total number of flies died during 168 h of observation ($n = 3$). $ indicates absence of Sh^5/OR and Sh^5/CS males due to Sh^5 being an X-chromosome mutation. Other abbreviations, culture and treatment conditions, *Drosophila* handling, etc. are as described in Table 1.

therefore ruled out the possibility that NaVP-induced differences in mortality rates of flies reflect differences in their capacity to withstand starvation. In brief, the mortality experiment showed that the drug is more toxic to OR, CS, Sh^3, Sh^6, Sh^{14} and $para^{ts1}$ than Sh^5. The *T*(half) of Sh^5/OR and Sh^5/CS F_1 individuals did not differ ($P > 0.1$) from that of Sh^5 (Table 2). This indicated the observed resistance of Sh^5 flies to the killing effect of NaVP to be a dominant character.

Among the four *Sh* mutants used in the study, molecular lesions are known for two, Sh^5 and Sh^{14} (syn. Sh^{KS133}) (ref. 11). In the topological model of *Sh* K^+ channel, Sh^5 and Sh^{14} missense mutations are located within the transmembrane segment S5 and the H5 bend region connecting the membrane spanning segments S5 and S6 respectively. Our results show that among the four strains, the two wild-types (OR and CS) and the two *Sh* mutants in which the molecular defects are known (Sh^5 and Sh^{14}), NaVP treatment brings down body weight of three (OR, CS and Sh^{14}), without affecting Sh^5. The common feature of OR, CS and Sh^{14}, as opposed to Sh^5, is that they have normal S5 segment. It is possible that K^+ channel activity required for body weight control is regulated through S5, a segment believed to interact with the voltage-sensing segment S4. The molecular lesions in Sh^3 and Sh^6 are unknown. Given our observation of these two mutants sharing NaVP-induced weight reduction property with OR, CS and Sh^{14}, it is tempting to speculate that Sh^3 and Sh^6 mutations affect functional component(s) of the *Sh* K^+ channel other than that defined by Sh^5 in segment S5. Unlike the wild-types OR and CS, the $para^{ts1}$ flies showed no reduction in body weight after NaVP treatment. This indicates that NaVP acts through *para* Na^+ channel and supports the hypothesis that it has voltage- and use-dependent Na^+ channel blockade activity. This also highlights the structural and functional conservation of Na^+ channel between fly and man. Studying the effect of NaVP on *Dsc*, the other *Drosophila* Na^+ channel structural gene and the other *para* alleles might provide useful information about NaVP pharmacology. The NaVP-induced mortality in OR, CS, Sh^3, Sh^6 and Sh^{14} suggested that death of flies might be caused by a loss in body weight. The effect of drugs on $para^{ts1}$ mortality however does not indicate so. If body weight loss resulted in the death of flies, the NaVP-induced mortality in $para^{ts1}$ should have been similar to that of Sh^5 as both of them are equally resistant to drug-induced body weight loss. Since this was not found to be the case, influence of NaVP on body weight and mortality seems to represent two separate effects of the drug. However, it is difficult to comprehend why Sh^5 is more resistant to VAL. This particular *Sh* allele directly or indirectly seems to interfere with the normal VAL toxicity. Sh^5 is a dominant mutation. The resistance to NaVP-induced lethality in heterozygotes (Sh^5/OR and Sh^5/CS) suggests the mutation to be dominant also with respect to the drug's toxicity.

In conclusion, we suggest an involvement of *Sh* K^+ channel in body weight control. It is interesting to note here that an ATP-sensitive K^+ (K_{ATP}) channel has been recently implicated in the regulation of food intake and body weight by the human and rodent hormone leptin[12]. Interestingly, instead of the six transmembrane organization of *Sh* K^+ channel, K_{ATP} channels possess only two transmembrane segments which appear to be homologues of the S5 and S6 helices of *Sh* channels. In addition, the antiparallel β-pleated strand, H5, of *Sh* channels shows sequence similarity to that of K_{ATP} channels[13]. The suggested role of K_{ATP} in body weight regulation is that it functions as the molecular end-point of the leptin signalling pathway. Our results indicate that NaVP influences *Sh* K^+ channels and affects body weight. Hence, understanding the pharmacology of NaVP in more detail might be useful in anti-obesity drug[14] research.

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Brassinosteroids and benzylaminopurine increase yield in IR50 indica rice

Brassinosteroids (BR) are naturally occurring steroidal plant growth regulators. Since the isolation of brassinolide from rape pollen in 1979, more than 40 natural analogs have been reported to be widespread in the plant kingdom[1]. In a variety of bioassays, BR are active at picomolar and nanomolar concentrations, as against micromolar concentrations of other plant growth regulators (PGRs). Physiological and molecular genetic studies support the view that BR should be considered as a new class of plant hormones[2–5]. BR promote elongation, division and differentiation of cells, and ethylene biosynthesis. They enhance auxin-mediated bending of rice leaf lamina[6] and growth of grass leaf sheath pulvinus[7]. External application of BR is reported to increase yield in vegetable and fruit crops and enhance resistance against salt, herbicides and fungal pathogens[8,9].

We investigated the effects of brassinosteroids and several other plant hormones and other PGRs on the vegetative and reproductive growth of IR50 rice. This report focusses on the promising results of yield increase obtained with BR and BAP.

Seeds of *Oryza sativa* var. indica cv IR50 were obtained from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. Rice plants were grown in pots in the nursery and in fields (2 × 2 m experimental plots) in the college farm. Desired concentrations of PGRs were prepared from stock as aqueous solutions containing a few drops of Tween 20 or Triton-X. The PGRs were applied as soaking spray treatments at various stages of development like vegetative, panicle initiation and anthesis[10]. The control plants were sprayed with water containing a few drops of the above detergents. Brassinolide was obtained from Zen-Noh, National Federation of Agricultural Cooperative Association, Tokyo, Japan. A synthetic isomer that gave similar results was supplied by W. J. Meudt of USDA, Beltsville, Maryland. All other PGRs used in this investigation were obtained from the Sigma Chemical Company, USA.

Periodic measurements were made on plants growing in the field and pot cultures. Fresh and dry weight of mature spikelets were determined for 1000 grains[11]. The data were analysed by the ranking of the treatment means. ANOVA Test and Duncan Multiple Range analyses were carried out to determine whether the results obtained were statistically significant. Free-hand

Table 1. Effects of PGRs on tillering, spikelet number, grain-filling and yield in IR50 rice†

Treatment molar (M)	Number of fertile tillers	%	Number of spikelets	%	Number of filled spikelets	%	Fresh weight of 100 grains (g)	%	Dry weight of 100 grains (g)	%
Control	8.0^{d}	–	74.30^{d}	–	63.70^{f}	–	2.316^{e}	–	2.158^{f}	–
Brassinosteroid (BR)10	13.5^{a}	68.7	110.40^{a}	48.5	104.6^{a}	64.2	2.853^{b}	23.2	2.728^{b}	26.4
Benzylaminopurine (BAP)10^{-5}	9.8^{c}	22.5	85.80^{c}	15.4	81.10^{e}	27.3	2.709^{c}	16.9	2.610^{c}	20.9
Gibberellic acid (GA_3)10^{-6}	6.6^{e}	−17.5	121.30^{a}	63.2	93.30^{c}	46.46	2.557^{d}	10.4	2.462^{e}	14.0
Kinetin (KIN) 10^{-5}	10.0^{c}	25.0	94.20^{b}	26.7	87.20^{d}	36.89	2.730^{c}	17.8	2.670^{b}	23.7
BR 10^{-7} + KIN 10^{-5}	11.9^{b}	48.7	97.40^{b}	31	91.90^{c}	44.27	2.842^{b}	23.0	2.738^{b}	26.8
BR 10^{-7} + BAP 10^{-5}	12.8^{a}	60.0	107.90^{a}	45.2	102.20^{a}	60.04	3.119^{a}	34.7	2.999^{a}	38.9
BR 10^{-7} + GA_3 10^{-6}	6.8^{e}	−15.0	121.40^{a}	63.4	89.0^{d}	39.7	2.717^{c}	17.3	2.538^{d}	17.6

%Promotion over control.
†Identical letters following the values indicate no significant difference according to Duncan Multiple Range Test ($P < 0.05$).